EFFECT OF *TRICHODERMA VIRIDE* AND RICE STRAW BIOCHAR ON THE DEVELOPMENT OF CHARCOAL ROT IN MAIZE

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ABSTRACT: The present study focused on the management of charcoal rot of maize caused by *Macrophomia phaseolina*, by using biochar made from rice straw, along with compost and *Trichoderma viride* (Biocontrol agent). Therefore, synergistic effect of 3 and 6 % concentrations of rice straw biochar with *T. viride* and 10 % of compost was used as a soil amendment to check the effect on growth of maize and charcoal rot disease suppression under glasshouse conditions. The percentage severity of charcoal rot was significantly reduced due to the effect of soil amendment with 3 % rice straw biochar inoculated with or without *T. viride*. *In vitro* assays also revealed synergistic association of rice straw biochar with *T. viride* and resulted into maximum inhibition (69.67 %) of *M. phaseolina*. The maximum nitrogen contents (20.26 g/kg) were recorded in rice straw biochar (3 %) combined with biocontrol agent amended treatment. However, other than disease parameters, 6 % rice straw biochar irrespective to the presence or absence of *T. viride* had a significant impact on growth of maize plants. The maize response were found to be dependent on the concentration of biochar (3 and 6 %) and depicted varied effects of resistance in each treatments. Thus, our findings suggest that the combined application of biochar and *T. viride* holds significant potential for mitigating the detrimental effects of charcoal rot and enhancing the overall health and performance of maize plants.

Key words: Disease Management, Organic carbon, *Macrophomia phaseolina,* Biocontrol agent, Plant protection

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INTRODUCTION

Agriculture has a crucial impact on the quality of life as it provides basic needs such as food, clothing, and shelter. Agriculture practices significantly impact climate changes, but extent of which vary from region to region. Meanwhile, an urgent requirement is that agriculture must adapt to modern lines to ensure food security for the rapidly growing Population (Anderson, Bayer and Edwards, 2020). Agriculture plays a significant role in the economies of developing countries like Pakistan, Bangladesh, India, and China. Pakistan's agriculture role in Gross Domestic Product (GDP) is 5.97% and hires around 50% among workforce, thus became the primary source of foreign exchange earnings (Pakistan Economic Survey, 2022).

Charcoal rot of maize is caused by *Macrophomina phaseolina* (Tassi) Goid., usually spread by both seeds and soil and affects more than 500 types of plants including maize to develop charcoal rot is one of the most significant diseases in terms of its economic impact. Due to the charcoal rot of maize, cob production has decreased about 18.7%, whereas losses in Pakistan and India range from 10 to 42% (Shekhar *et al.*, 2006). Infected plants show wilting symptoms. Earlier symptoms in plants with disease includes grey line on their stalks. The pith starts to fall off, and tiny, greyish-black sclerotia begin to appear on the vascular bundles. Frequently, the stalk's interior is peeled apart, which leads to crown-area stalk breaks. Later signs show black coloring that appears on the crown of the affected plants (Xavier and Kaushik, 2021). Primary source of infection is from infected plant debris and seeds or micro-sclerotia in soil. *Macrophomina phaseolina* in soil survive up to 15 years as a saprophyte (Kaur *et al*., 2012).

Biological control is a sustainable approach to manage plant pathogens without using harmful chemicals (Selari *et al.*, 2023). Several microorganisms were reported to have potential to serve as biocontrol agents against *M. phaseolina,* which includes *Trichoderma* spp., *Bacillus* spp., *Pseudomonas fluorescens*, and several fungal antagonists such as *Gliocladium virens*, *Coniothyrium minitans*, and *Aspergillus flavus* (Yadav and Meena, 2023). These microorganisms can colonize the rhizosphere of plants and compete with *M. phaseolina* for nutrients and space (Sharma, 2023). *Trichoderma viride* is a species of fungi commonly used as a biological control agent against plant pathogens. Due to naturally occurring in soil the fungus has the ability to produce various enzymes and secondary metabolites that can inhibit the growth of pathogenic fungi and bacteria (Harikrishnan *et al.*, 2023). Among the main mechanisms of *T. viride* to control plant pathogens induced damages is by competing for space and nutrients(Li *et al*., 2023). *Trichoderma viride* also enhances the plant growth and ability to suppress diseases could be attributed to production of antifungal compounds (chitinases and glucanases) with the potential to damage the cell wall of fungal pathogens (Nujthet, Jantasorn and Dethoup, 2023).

Organic matter in the soil increases the number of beneficial microbes which can interfere with the development of *M. phaseolina*. Soil amendments with crop wastes, biochar or compost promote soil health and plant defense (De Corato, 2023). Biochar is a charcoal type material, made by burning organic feedstock, e.g. wood, leaves, and other organic wastes, in the little or absence of the oxygen, method called pyrolysis, which converts the organic matter into carbon rich matter (Downie, Crosky and Munroe, 2012). Biochar balance the soil pH, thereby creating a more favorable environment for plant growth and mitigating the impact of soil-borne pathogens, attributed to the alkaline properties of biochar, which counteract soil acidity, and the resultant changes in soil chemistry and microbiology that can promote plant health and resilience (Liao and Thomas, 2019).

So, the study was carried out with the following goals in order to determine the impact of biochar, compost, and biological control agent on charocal rot of maize: (a) to estimate the effect of biochar on maize growth and charcoal rot incidence and severity, (b) to study the synergistic response of biochar and *Trichoderma viride* on the plant growth and disease suppression in maize, (c) to assess the *in-vitro* antimicrobial potential of biochar and *T. viride* individually and in combination against *Macrophomina phaseolina* and (d) to analyze the impact of *T. viride* in mitigating charcoal rot disease of maize.

MATERIALS AND METHODS

Preparation of biochar: Raw material comprising of rice (*Oryza sativa*) straw was collected from the fields of Department of Plant Pathology, (31. 29′ 42.2664″ N, 74. 17′ 49.1316″ E,) Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan to process for biochar production. Biochar was made by pyrolyzing (rice straw) at the temperature of 400℃ (Liu *et al*., 2022). The top lit updraft (TLUD) portable kiln method was used after applying minor modifications to make biochar due to its low cost and good efficiency (Mohammadi *et al.*, 2017). The whole apparatus is portable and consist of three main parts A) Main container or Drum, B) After container or Adopter and C) Chimney (Aftab *et al.*, 2022).

Soil Preparation: Organic compost was mixed thoroughly at the rate of 10% in the sterilized soil. The soil was taken as the primary substrate for producing distinct potting mixtures, incorporating biochar at concentrations of 3% and 6% (v/v). These potting mixtures, augmented with biochar, were utilized for plant cultivation purpose (Yang *et al.*, 2020).

***Macrophomina phaseolina* and *Trichoderma viride* culture acquisition:** *Macrophomina phaseolina* and *Trichoderma viride* cultures were taken from the First Fungal culture bank of Pakistan (FCBP), Department of Plant Pathology, University of the Punjab, Lahore.

Multiplication of pathogen and biocontrol agent: Potato dextrose agar (PDA) medium was formulated to facilitate the multiplication of the fungal pathogen (*Macrophomina phaseolina*), and biocontrol agent (*Trichoderma viride*).

Characterization of the *Macrophomina phaseolina* and *Trichoderma viride*: Macroscopic and microscopic observations were taken to characterize the morphology of *M. phaseolina* and biocontrol agent (Mallikarjuna & Jayapal, 2015).

Preparation of bio control agent suspension: A solution containing conidia (asexual spores) of *Trichoderma viride* was prepared using a seven-day-old culture of the fungus. The culture was poured with 10 mL of sterilized distilled water, and the fungal biomass was gently scraped off using a sterile glass slide. The resulting suspension was then filtered through cheese cloth to remove any large debris. The concentration of *T. viride* conidia in the suspension was adjusted to 1×10^6 conidia per mL was determined by using spectrophotometer at optical density of 550nm (Waghunde *et al.*, 2010).

***In vitro* effect of rice straw biochar and *Trichoderma viride* on *Macrophomina phaseolina* growth:** In the present study, the *in vitro* assessment of *M. phaseolina* was conducted by using *T. viride* in PDA media plates. To check biochar effect on fungal growth, 3% biochar (V/V) biochar was added in the media Petri plates. Plates were incubated at 37 ^{0}C in an incubator for 5 days. After every 24 hours of incubation readings were taken up to 3 days. Percentage growth inhibition (PI) was calculated as follow (Khan & Javaid, 2020).

$$\mathbf{PI} = \frac{\text{Control}-\text{Treatment}}{\text{Control}} \times 100$$

Experimental design: Maize and Millets Research Institute variety seeds (MMRI yellow) of maize were taken from the Federal Seed Certification and Registration Department, Lahore. Seeds were surface sterilized with 4% sodium hypochlorite or house holding bleach (NaOCl) before sowing and washed three times thoroughly with distilled water before sowing in pots (Pal *et al*., 2022). To explore the potential effects of rice straw biochar, pots were filled by using of 3 and 6% rice straw biochar concentrations. These additions were made with the aim of investigating the effects of biochar on maize and *M. phaseolina*, both in the presence and absence *T. viride.*

Inoculation of *T. viride* in soil: Soil inoculation was done with *T. viride* with about 7 to 10 days old culture. Suspension of 50 mL was inoculated in each bio control treatment pots. Only in the bio control containing treatments, maize seeds were subjected to pre-sowing immersion in a spore suspension of *T. viride,* 1×10^6 spores per mL concentration. Subsequently, the treated seeds were sown in the bio control treatment pots for further experimentation (Garg, Singh and Prakash, 2007).

Plant inoculation with *M. phaseolina:* The inoculations were conducted on 15-day-old maize plants. Fresh and pure cultures of the fungal pathogen, *M. phaseolina*, were utilized, and the concentration of conidia was determined under a compound microscope. For the inoculation procedure, a needle was inserted to a depth of approximately 2 mm into the stems of the maize seedlings. Each seedling received 1 mL inoculum containing 50 pycnidia per mL, which was carefully introduced into the stem (Shehbaz *et al.*, 2018). Moreover, soil inoculation of the pots was conducted by adding 50 mL of a suspension containing 1×10^5 microsclerotia per mL at the basal region of each pathogen inoculated plant (Pastrana *et al.*, 2016).

Maize physiological parameters assessment: The plants were grown under controlled conditions in a glass house to ensure uniformity in growth and environmental factors. Adequate water, light, and temperature conditions were maintained throughout the duration of experiments. To check the effect of biochar, biocontrol and pathogen on maize plants the shoot length, root length, shoot weight and root weight was measured.

Chlorophyll estimation: For chlorophyll contents, leaf samples were collected from each treatment group at the onset of the tasseling stage in maize. Subsequently, optical density (OD) values of the samples were recorded at two specific wavelengths, 663 nm and 645 nm, using a spectrophotometer. Pure acetone was used as a blank reference, and chlorophyll a, chlorophyll b, and total chlorophyll contents were determined based on these measurements (Dash *et al.*, 2022). The amount of total chlorophyll (mg/g fresh weight of leaf) was calculated by using the following formulae.

Total chlorophyll contents = chlorophyll A + chlorophyll B

Nitrogen [N], phosphorus [P] and potassium [K] contents determination: For NPK quantification, maize leaves from each treatment were obtained and sun dried sun dried where, they were exposed to a specific temperature for a predetermined duration, facilitating the complete evaporation of moisture from the plant tissues (Wang *et al*., 2018). Total nitrogen in leaf samples was checked by using Kjeldhal Method (Keeney and Bremner, 1966). Potassium contents were checked by using flame emission spectrophotometry (Estefan, Sommer and Ryan, 2013). The potassium (K) content g/kg was determined using a flame photometer, with standard solutions used for calibration and readings obtained from a calibration graph.

Disease Assessment: Evaluation of charcoal rot disease on maize was done after 60 days of sowing. A disease rating scale ranging from 0-5 on the basis of disease intensity from lowest to severe was used to assess the impact of treatment on disease intensity (Siddique *et al.*, 2021). To determine the disease incidence and percent severity index following formulas were used;

$$\text{Disease Incidence (\%)} = \frac{\text{Number of plants affected by the disease}}{\text{Total number of plants in the sample}} \times 100$$

Whereas,

$$\text{Percent Severity Index (PSI \%)} = \frac{\text{Sum of Individual Severity Ratings}}{\text{Maximum Possible Severity Rating x Total Number of Plants}} \times 100$$

Furthermore, disease response was meticulously examined for each treatment group subjected to pathogen inoculation. The response of the plants to the disease was recorded and described based on the disease rating grades.

Statistical analysis: The statistical analysis was performed using Statistics 8.1 software. To determine uniformity and detect significant differences among means, the Tukey HSD (Honestly Significant Difference) All-Pairwise Comparisons test was employed at a significance level of $P < 0.05$. The experimental design used for the analysis was a completely randomized design.

RESULTS

Characterization of the *Macrophomina phaseolina*: Sclerotia of *M. phaseolina* exhibit an irregular shape, with the exception of a few specimens that exhibit a round to oblong morphology. The mycelium of the fungus was characterized by septate hyphae measuring approximately 1.5 to 2.5 micrometers in width. Initially hyaline in color, the mycelium undergoes a transition to a honey or black hue over time. Microsclerotia of *M. phaseolina* exhibit a characteristic black coloration, and their size demonstrates variability both on infected plants and in laboratory media. Specifically, under laboratory conditions, the sclerotia display a hyaline to light brown coloration. In natural conditions, the diameter of the sclerotia ranges from 50 to 120 micrometers.

Characterization of biocontrol agent: The following features were commonly examined: The hyphae of *T. viride* were examined. These hyphae typically exhibited a long, slender appearance and were divided into compartments known as septa. The color of the hyphae ranged from colorless to having a greenish hue, attributed to the production of pigments. The presence of conidiophores, specialized structures responsible for producing conidia (asexual spores), was assessed. Conidiophores of *T. viride* were often observed to be branched and carried conidia at their tips. Conidia themselves displayed typical characteristics, appearing green in color and exhibiting a spherical or oval shape.

Maize Plant growth assessment: The examination of various factors, such as rice straw biochar and *T. viride*, revealed positive effects on the growth parameters of maize. These parameters encompass the length and weight of the shoots and roots of the maize plants. However, a noteworthy effect was observed among the soil composition, compost application, and presence of *T. viride*, exerting a substantial influence on the production of both above and belowground plant biomass, as well as the elongation of root and shoot structures. The concentration of rice straw biochar in soil has a discernible impact on the overall growth of plants, characterized by the suppression of pathogens and the facilitation of plant growth.

Shoot length: The maximum maize shoot length was observed (38 and 46 cm) in 3% rice straw biochar amended treatment (S+3%RB) in the presence (+MPH) or absence (-MPH) of *M. phaseolina*, respectively (Figure 1). Nevertheless, the pathogen-inoculated plants (+MPH) displayed the minimum shoot length, measuring 25 cm in the treatment (S+MPH+BCA), while the un-inoculated plants (-MPH) exhibited a shoot length of 26 cm in the treatment 'S+6%RB+BCA'. The presence of disease resulted in stunted plant growth and a reduction in height. Additionally, a higher concentration of biochar, specifically 6%, did not guarantee the increase in shoot length.

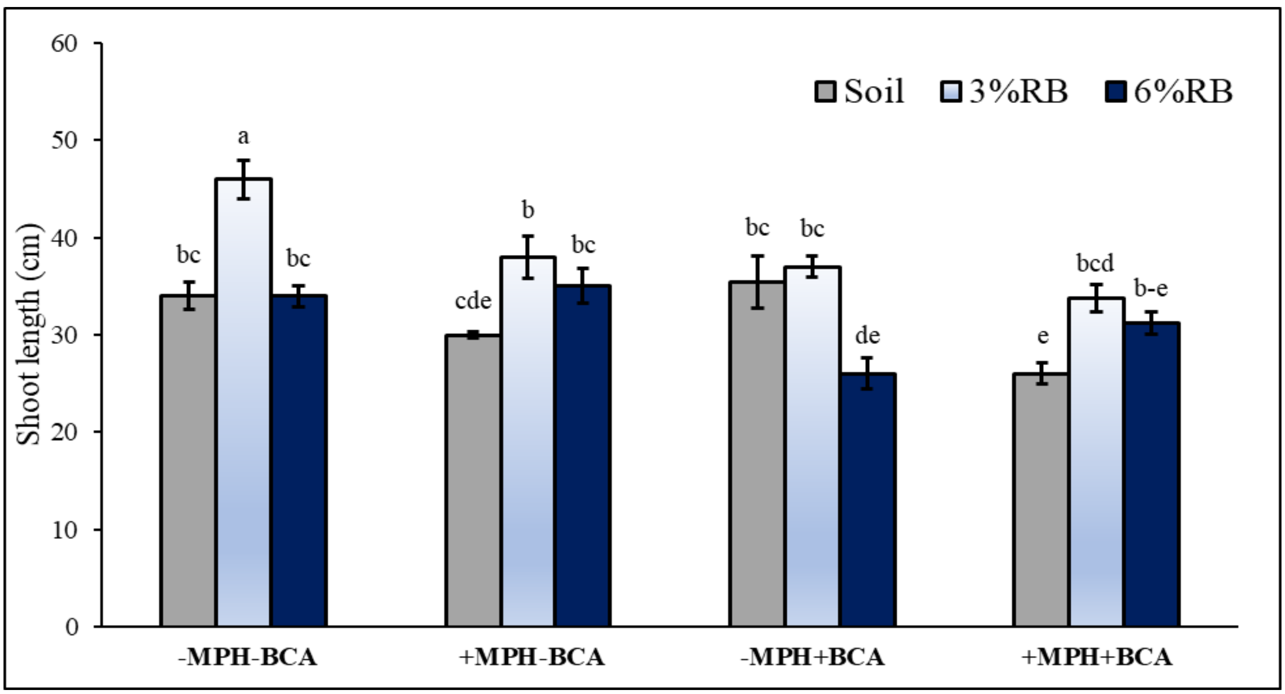

Figure 1: Effect of rice straw biochar (RB) (3% and 6%) on maize shoot length, together with *Trichoderma viride* (+BCA) and without (-BCA) biocontrol agent, either inoculated (+MPH) or un-inoculated (-MPH) with *Macrophomina phaseolina*.

Root length: In the absence of the pathogen *M. phaseolina*, the treatment with 3% rice straw biochar amendment (S+3%RB) exhibited the maximum maize root length, measuring 37 cm. Conversely, the presence of pathogen inoculation (+MPH) had only marginal effects on root length (Figure 2). The minimum root length 20 cm was in the treatment 'S+MPH', while the (-MPH) plants exhibited a root length of 26 cm in the treatment amended with 6% biochar together with *T. viride*. The presence of disease stress had an inhibitory effect on root growth. Moreover, higher concentration of biochar, specifically 6%, was associated with an increase in root length, in pathogen inoculated plants but in the absence of biocontrol agent.

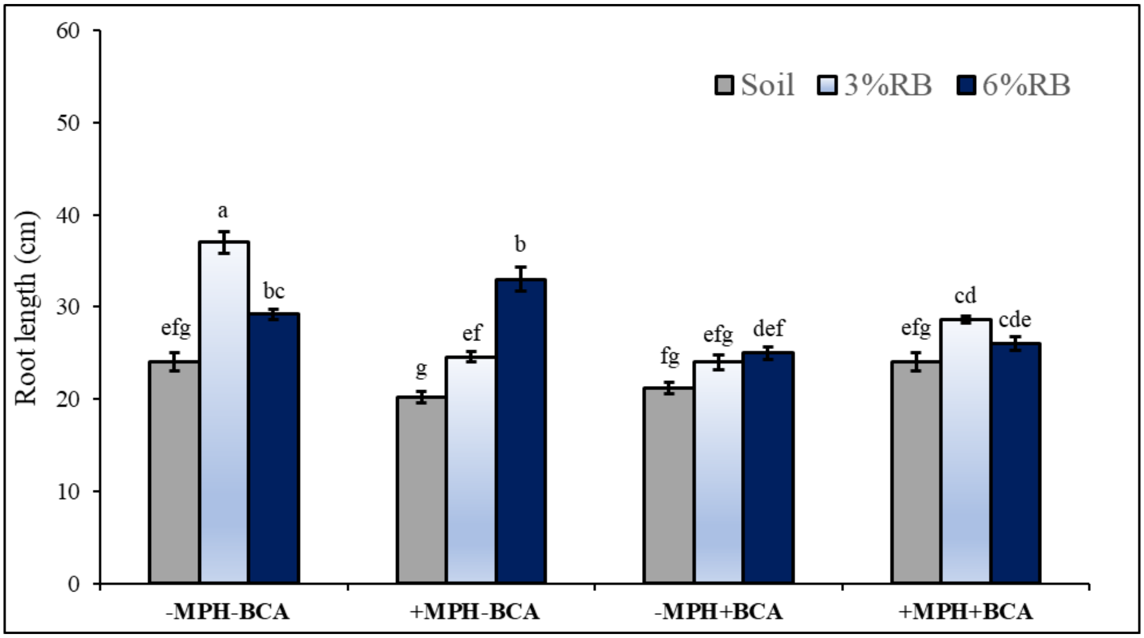

Figure 2: The influence of rice straw biochar (RB) (3% and 6%) on maize root length in the presence of (*Trichoderma viride*; +BCA) or absence (-BCA) of a biocontrol agent, either inoculated (+MPH) or un-inoculated (-MPH) with *M. phaseolina*.

Shoot weight: The highest maize shoot weight 23g was recorded in 6% rice straw biochar amended treatment without *M. phaseolina* (-MPH) (Figure 3). However, the pathogen-inoculated plants (+MPH) exhibited the lowest shoot weight, measuring 10 g, in the treatment 'S+MPH'. Furthermore, the second-highest weight 19.6 g was recorded in the treatment (S+3%RB). The presence of disease stress overall resulted in lesser shoot weights. Overall biochar, concentrations (3% and 6%), contributed to maximum weight efficiency only with (+BCA) as 18 g in (Soil + 6%RB + BCA) and 17.8 g in (Soil+ 3%RB + BCA) in absence (-MPH) of pathogen, but in presence (+MPH) of pathogen only 6% biochar showed better impact in shoot weight as 15.4 g in (Soil+ 6%RB + Pathogen + BCA) and 13.8 g in (Soil + 6%RB + Pathogen) respectively.

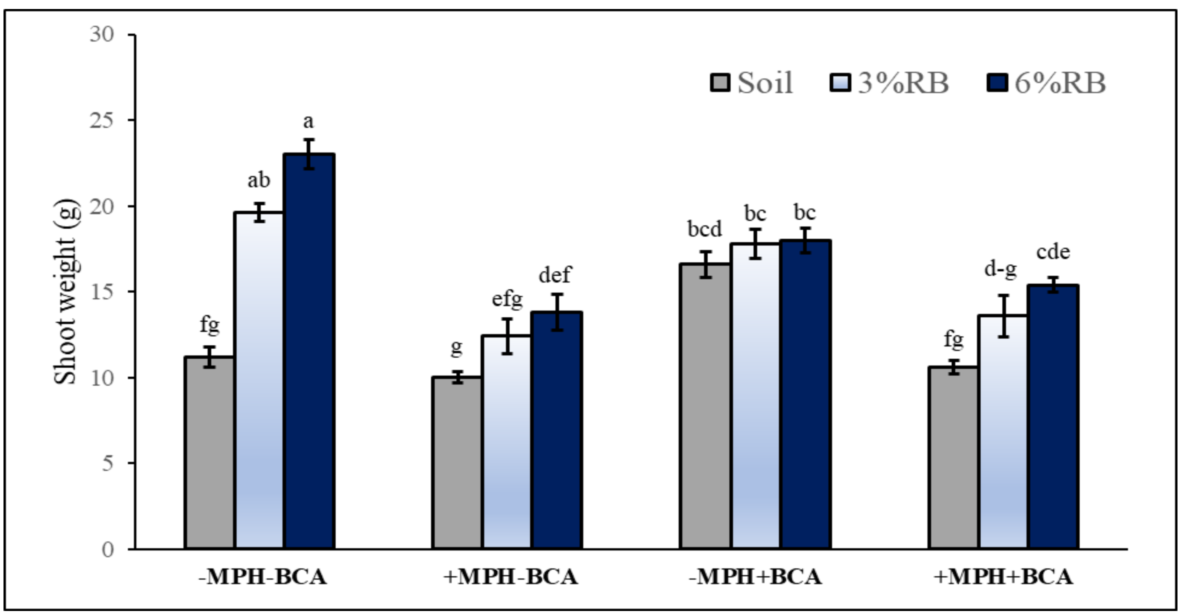

Figure 3: The impact of rice straw biochar (RB) (3% and 6%) on maize shoot weight examined under different conditions, including the presence (+BCA) or absence (-BCA) of a biocontrol agent, either inoculated (+MPH) or uninoculated (-MPH) with *M. phaseolina.*

Root weight: Application of 6% rice straw biochar amendment (S+6%RB) resulted in the highest maize root weight under pathogen-free conditions without *M. phaseolina*, measuring 5.08 g while, the presence of the pathogen (+MPH) led to a decrease in root weight. The treatment 'S+MPH' exhibited the lowest root weight, measuring 0.87g. Additionally, the second-highest weight of 4.69 g was recorded in the treatment amended with 6%RB and biocontrol agent. (Figure 4). Significantly, 6% biochar contributed to maximum root weight with (+BCA) as 4.02 g (Soil + 6%RB + Pathogen) in the presence (+MPH) of pathogen.

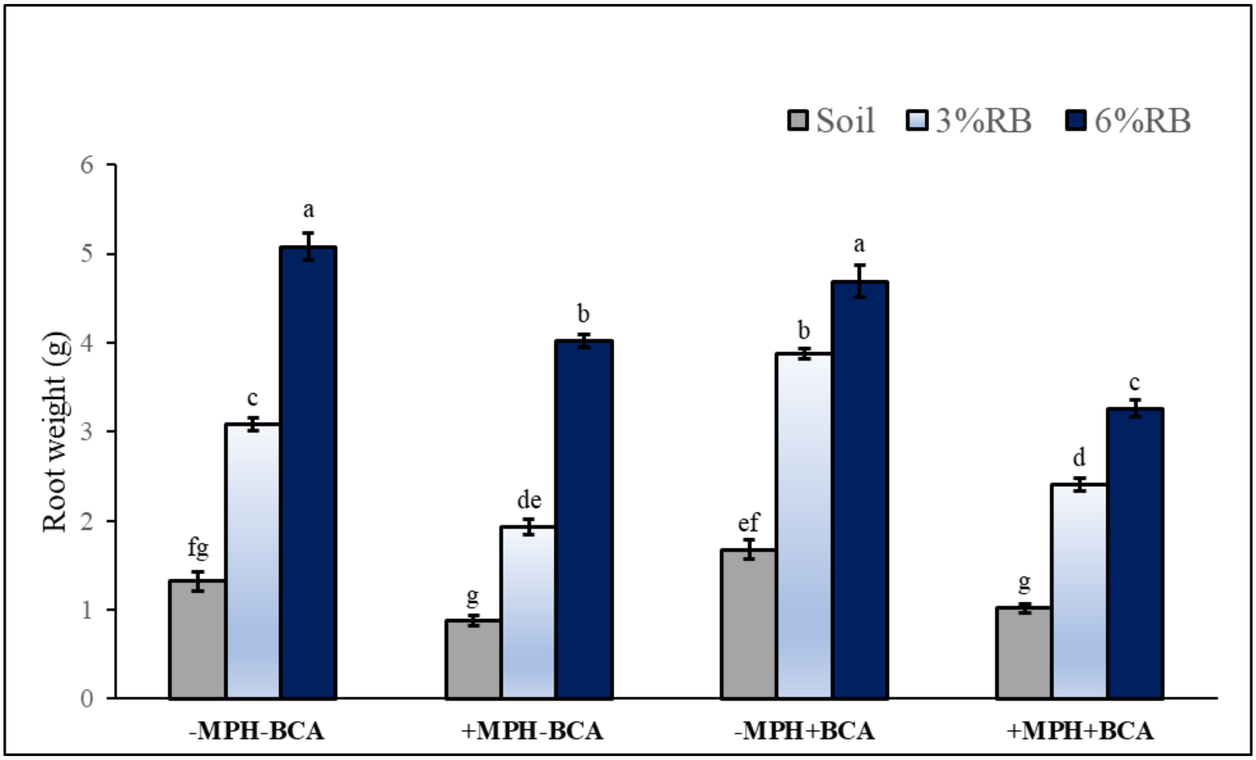

Figure 4: The influence of rice straw biochar (RB) (3% and 6%) on maize root weight was investigated in the presence (+BCA) or absence (-BCA) of a biocontrol agent, either inoculated with (+MPH) or un inoculated (-MPH) with *M. phaseolina*.

Effect of rice straw biochar on maize leaf chlorophyll contents, dry weight of shoot and root biomass: In terms of shoot and root dry weight, the treatment that showed the highest weight was 'Soil+6%RB', with a dry shoot weight of 7.03±0.15 and dry root weight of 1.79±0.15g. This treatment significantly outperformed then others in terms of dry weight of plant biomass. On the other hand, the treatment with the lowest shoot and root dry weight was 'Soil+MPH' which resulted in a comparatively lowest dry weight of 2.33±0.15 and 0.28±0.07g respectively. Moreover, the 3%RB also show effective dry shoot weight of 6.7±0.26 grams, but also the 6%RB with BCA showed the second highest dry root weight of 1.63±0.03g (Table 1).

Significantly, the 6% RB demonstrated the good dry shoot and root weight efficiency in treatment (Soil+6%RB+BCA) with biocontrol agent (+BCA) of 6.63±0.05 and 1.63±0.13 respectively.

Regarding total chlorophyll content, the treatment with the highest chlorophyll content was 'Soil+3%RB+MPH, exhibiting a maximum chlorophyll content of 0.40 µg/mL (Table 1). This treatment demonstrated a significant increase in chlorophyll levels, indicating better photosynthetic activity due to 3%rice straw biochar mixed in the soil. The biochar is known to be most effective for maintaining the soil structure for plant growth and development. In contrast, the treatment with the lowest chlorophyll content was Soil+MPH, with a value of 0.33 µg/mL. This treatment showed lower chlorophyll production due to pathogen presence without any treatment amended in soil showed reduced photosynthetic efficiency. Overall, the treatment of Soil+6%RB consistently demonstrated favorable results across all parameters, with the highest shoot and root dry weights. Additionally, Soil+3%RB+MPH exhibited the highest chlorophyll content, indicating efficient photosynthesis. These findings suggest that the addition of rice straw biochar (RB) at 6% concentration in the soil may have positive effects on plant growth, while the combination of *Macrophomina phaseolina* (MPH) and

Trichoderma viride (BCA) might not be as effective in enhancing plant development.

Table 1: Effect of rice straw biochar on maize leaf chlorophyll contents, dry weight of shoot and root biomass.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Total chlorophyll contents (µg/mL)
Soil Only	2.58±0.11^{de}	0.67±0.02^{e}	0.39
Soil+MPH	2.33±0.15^{e}	0.28±0.07^{f}	0.339
Soil+BCA	4.95±0.05^{c}	0.42±0.03^{ef}	0.34
Soil+MPH+BCA	2.45±0.06^{e}	0.32±0.02^{f}	0.38
Soil+3%RB	6.7±0.26^{ab}	1±0.1^{d}	0.39
Soil+3%RB+MPH	2.65±0.05^{de}	0.47±0.02^{ef}	0.40
Soil+3%RB+BCA	5.12±0.10^{c}	1.37±0.28^{bc}	0.39
Soil+3% RB+MPH+BCA	2.9±0.1^{d}	0.62±0.02^{e}	0.39
Soil+6%RB	7.03±0.15^{a}	1.79±0.15^{a}	0.38
Soil+6%RB+MPH	2.71±0.13^{de}	1.47±0.03^{b}	0.39
Soil+6%RB+BCA	6.63±0.15^{b}	1.63±0.03^{ab}	0.38
Soil+6%RB+MPH+BCA	5.19±0.06^{c}	1.53±0.13^{cd}	0.39

[Nitrogen (N), Phosphorus (P) and Potassium (K)] contents of Maize plant: Nitrogen is a crucial nutrient for plants, playing a vital role in genetic and metabolic processes that support plant growth and crop productivity. The impact of soil amendments, including biochar, compost, and biocontrol agents, on the nutrient content and uptake of maize shoots was recorded. Figure 5 demonstrated the decline in nitrogen contents in diseased plants as the lowest nitrogen (12 g/kg) was recorded in maize grown in pathogen inoculated soil (+MPH). Significantly the 3% rice straw biochar amended with biocontrol agent in the soil (Soil+3%RB+BCA) showed maximum nitrogen contents as 20.26 g/kg in maize plants. Moreover, the plants with 6% rice straw biochar in the presence of (+BCA) biocontrol agent (Soil+6%RB+BCA) showed the second highest 20 g/kg nitrogen contents in maize plants. Overall both 3 and 6% rice straw biochar are efficient in the presence of (+BCA) for nitrogen uptake, while the treatments with 3% biochar showed the maximum nitrogen contents with '+BCA'.

Our results had shown that the phosphorus content in maize shoots varied across different treatments (Figure 6). The range of total phosphorus values observed was between 1.78 and 3.15 g/kg. The highest significant value of phosphorus was observed as 3.15 g/kg in 3% rice straw biochar (RB) in following treatment (S+3%RB), without pathogen and biocontrol agent (-MPH/-BCA). The highest value among biocontrol agent containing treatments was 2.99 g/kg, which corresponds to the treatment 'S+3%RB+BCA'. This treatment involves the presence of the biocontrol agent (BCA) along with 3% rice straw biochar (RB), but without the pathogen (-MPH). While, the lowest value of 2.36 g/kg of phosphorous contents, was found in the treatment 'S+6%RB+BCA'. The lowest phosphorus content value among all the treatments is 1.78 g/kg, which corresponds to the treatment "S+MPH" (soil with plant and pathogen).

In case of Potassium (K) content significant differences in phosphorus contents were observed among different treatments (Figure 7). As a result of pathogen inoculation (+MPH) maize plants in treatment 'S+MPH' showed the lowest potassium contents (7.66 g/kg). In contrast, the application of the biocontrol agent (+BCA) in (S+BCA) resulted in a potassium content of 11.00 g/kg. This suggests that the presence of the biocontrol agent positively influenced potassium uptake in the absence of other amendments. The treatment with 3% RB amendment in soil, without the pathogen and biocontrol agent (-MPH/-BCA) demonstrated the maximum potassium contents as 15.66 g/kg. The elevated potassium content observed in plants can be attributed to the presence of potassium-rich compounds present in rice straw biochar. However, increasing the RB concentration to 6% did not exert a notable influence on potassium availability or uptake, in comparison with 3% RSB amended treatment. Treatment 'S+3%RB+MPH+BCA' has 14.16 g/kg of potassium contents, while the treatment with the combined presence of the pathogen, biocontrol agent, and 6% RB (S+6%RB+MPH+BCA) exhibited a remarkably low potassium content of 8.93 g/kg.

Disease Assessment: By comparing all the treatments for the results showed in (Table 2) that the treatment 3 (Soil + 3%RB + MPH) have shown the best control of charcoal rot disease with the lowest disease incidence. Whereas, the treatment 2 and 4 (Soil + MPH + BCA) and (Soil + 3%RB + MPH + BCA) provide moderate control over the disease with respect to other treatments. However, the treatments 5 and 6 (Soil + 6%RB + MPH) and (Soil + 6%RB + MPH + BCA) demonstrate the highest susceptibility and severity. This suggests that the 6%RB is not effective in controlling the charcoal rot disease, but the rice straw biochar used with lower quantity percentage as 3% is most effective in controlling charcoal rot disease, moreover 3%RB addition with biocontrol (BCA) is also effective. Significantly, only soil with

'BCA' inoculated with pathogen have displayed the effectiveness in controlling charcoal rot disease than the treatment amended with 6% RB.

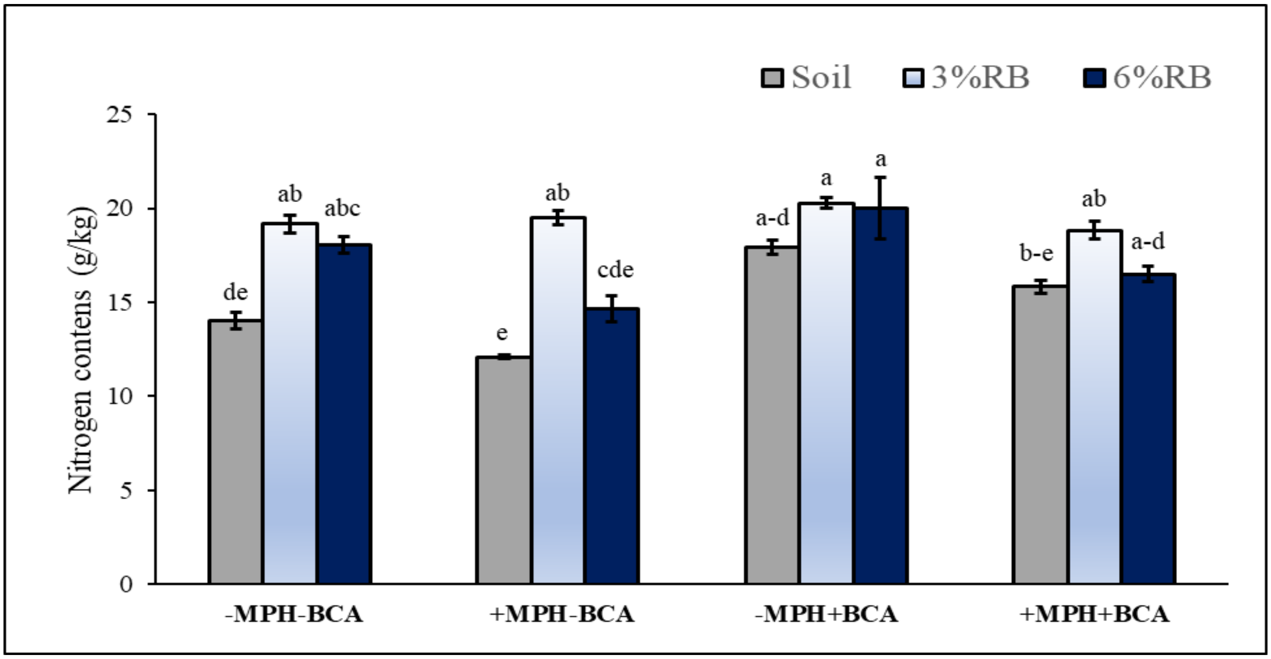

Figure 5: Effect of rice straw biochar (RB) (3% and 6%) on nitrogen contents in maize plant with *Trichoderma viride* (+BCA) and without (-BCA) biocontrol agent, either inoculated (+MPH) or un-inoculated (-MPH) with *M. phaseolina*.

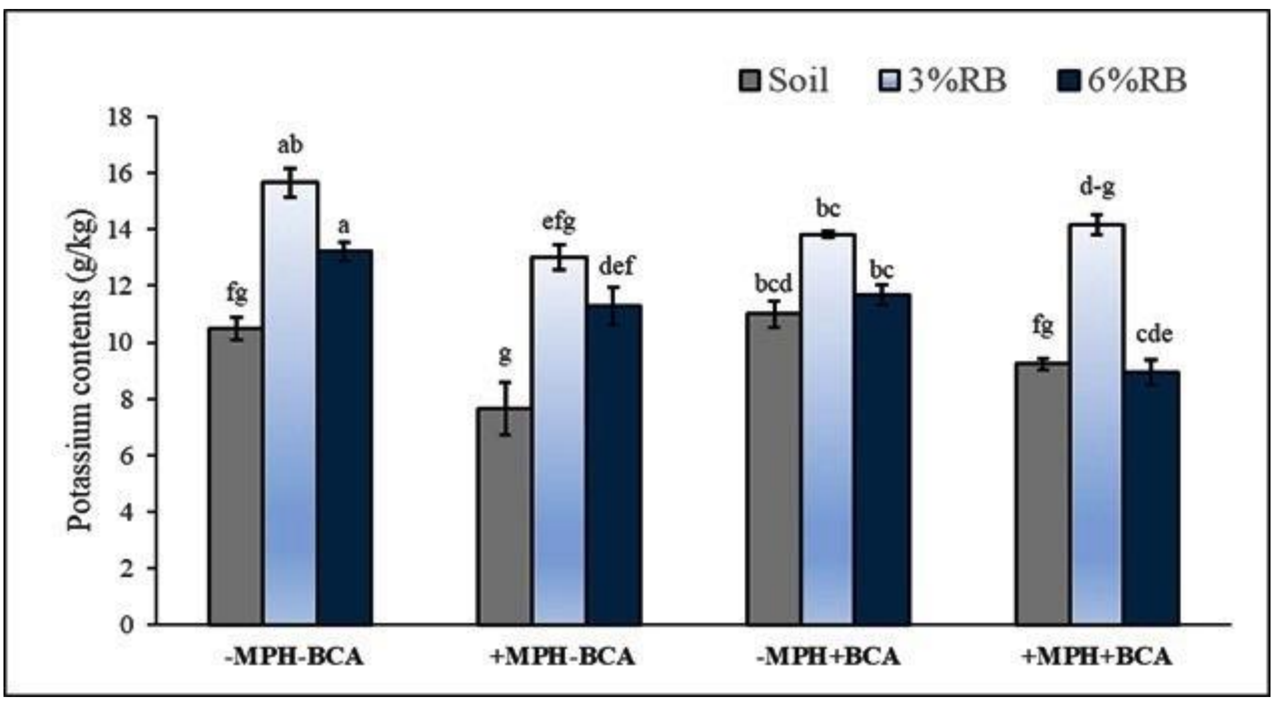

Figure 6: Impact of rice straw biochar (RB) (3% and 6%) on phosphorus contents (g/kg) in maize plants with in the presence (+BCA) or absence (-BCA) of a biocontrol agent, either inoculated with (+MPH) or uninoculated (-MPH) with *M. phaseolina*.

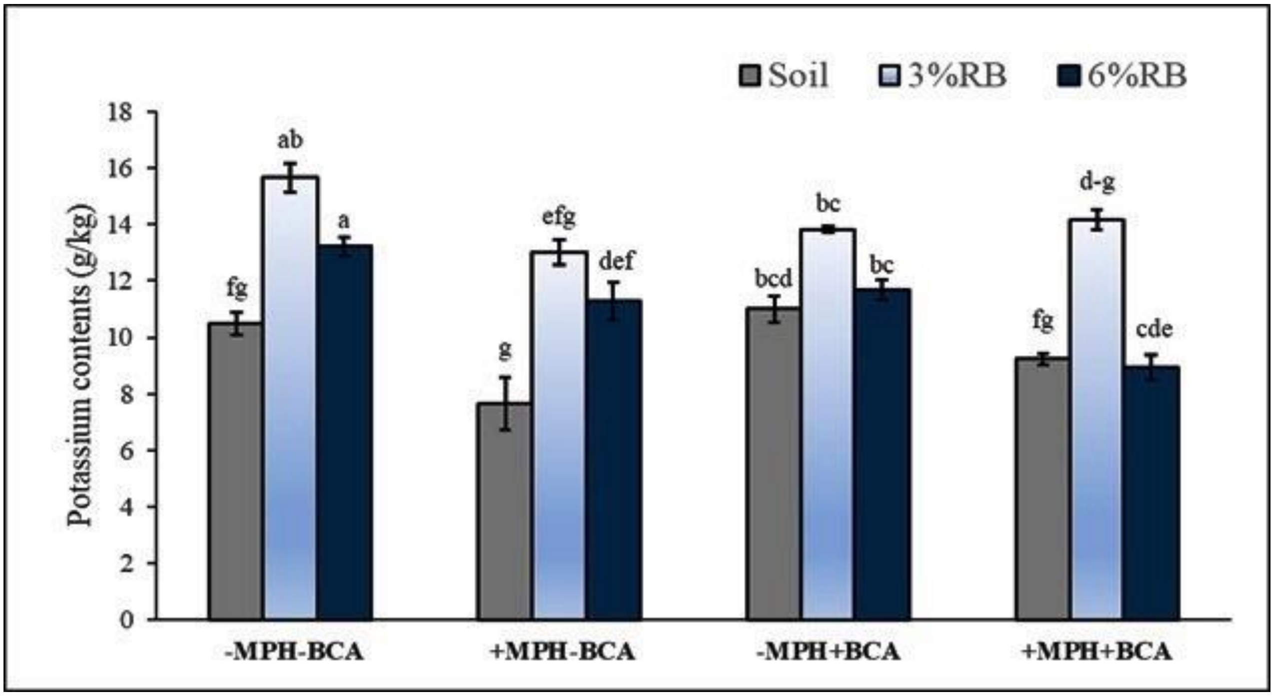

Figure 7: Impact of rice straw biochar (3% and 6%) on potassium levels of maize crop along with or without the effect of biocontrol agent (+BCA, -BCA, respectively) either inoculated with pathogen (+MPH) or un-inoculated (-MPH) plants.

Table 2 Disease rating scale for charcoal rots disease assessment

Treatments	Disease Incidence (DI %)	Percent severity Index (PSI %)	Disease Response
Soil + MPH	60	84^{ab}	Highly Susceptible
Soil + MPH + BCA	40	64^{d}	Susceptible
Soil + 3%RB + MPH	20	40^{e}	Moderately Susceptible
Soil + 3%RB + MPH + BCA	60	44^{e}	Moderately Susceptible
Soil + 6%RB + MPH	100	80^{bc}	Susceptible
Soil + 6%RB + MPH + BCA	80	88^{a}	Highly Susceptible

***In vitro* effect of rice straw biochar with *Trichoderma viride* on *Macrophomina phaseolina*:** Based on the results in (Table 3), it can be observed that all treatments (T_2, T_3, and T_4) showed some level of inhibition of *Macrophomina phaseolina* compared to the control (T_1). The percentage inhibition indicates the extent of control achieved by each treatment (Table 3). Treatment T_4 (MPH+BCA+RB) exhibited the highest inhibition with approximately 69.67%, whereas, treatment T_3 (MPH+BCA) demonstrated lower inhibition of 40.98%. It means that inhibition of pathogen 'MPH' by biocontrol agent (BCA) is more effective in the presence of rice straw biochar, meanwhile using only BCA also inhibited the (MPH). The treatment T_2 (MPH+RB) resulted in 16.39% inhibition of 'MPH' by rice straw biochar (RB) and depicted the lowest percentage of inhibition. By comparing treatment T_4 (MPH+BCA+RB) with treatment T_2 (MPH+RB), treatment 4 shows more promising results in inhibiting the growth of MPH. The percentage inhibition for T_4 is significantly higher than that of T_2, suggesting a more pronounced effect on suppressing the growth of the pathogen. Our results showed that the combination of the MPH with BCA and rice straw biochar results has more consistent and reliable inhibition of *M. phaseolina* growth compared to the treatment with only rice straw biochar.

Table 3: *In vitro* effect of rice straw biochar with *Trichoderma viride* on *Macrophomina phaseolina.*

Treatments	Replicates	3 DAI (cm)	Mean 3 DAI	SD	Percent inhibition (%)
T_1 (Control)	R_1	4	4.06	0.11	
	R_2	4.2			
	R_3	4			
T_2 (MPH+RB)	R_1	4	3.4	0.52	16.39[c]
	R_2	3			
	R_3	3.2			
T_3 (MPH+BCA)	R_1	2.6	2.4	0.62	40.98[b]
	R_2	2.9			
	R_3	1.7			
T_4 (MPH+BCA+RB)	R_1	1	1.23	0.25	69.67[a]
	R_2	1.5			
	R_3	1.2			

DISCUSSION

In the modern era of agriculture, the chemical management of diseases through the application of fungicides has emerged as the predominant and enduring strategy (Bakade, Sundaresha and Lal, 2022). Ons *et al*. (2020) reported that pathogen has ability to make itself resistant for repeated chemicals. Nevertheless, the widespread application and improper utilization of fungicides have resulted in emergence of fungicide resistance, exacerbated to ecological problems (Behera *et al.*, 2022). Hence, the present investigation was undertaken to devise an innovative, sustainable, and economically viable methodology for managing charcoal rot disease to mitigate pathogen-induced losses without compromising the biodiversity of the surrounding environment. Our results showed that 3% rice straw biochar was effective in root and shoot development of maize plants even in the presence of charcoal rot disease. Use of biochar in soil lead to increase the water and nutrient holding capacity of the soil, as well as improve soil structure, which can lead to better root growth and nutrient uptake. As a result plants become more resistant to stresses such as drought, nutrient deficiency, and diseases (Joseph *et al.*, 2021). Kumar & Bhattacharya. (2021) showed that the use of biochar in soil lead to healthier plants with increased resistance to stresses and diseases, making it a promising tool for sustainable agriculture.

In vitro analysis showed that *T. viride* along with rice straw biochar was more effective in controlling *M. phaseolina.* Hossain *et al*. (2020) studied that biochar is known to have high surface area, thus serving as a good absorbent for nutrients and moisture as well as provide space for microbial growth. Asghari *et al*. (2023) reported that the co-application of biochar and *Trichoderma* in heavy metal-contaminated soils resulted in enhanced growth of sainfoin seedlings. This enhancement was attributed to improved physiological parameters in the leaves, increased root growth, and alleviation of oxidative stress-induced damage Cao *et al*. (2022) revealed that using biochar with *Trichoderma* enhanced the growth of cucumber especially of the roots.

Our data suggested that the 3% rice straw biochar amended with *T. viride* in the soil showed maximum nitrogen contents in maize plants. Li *et al*. (2022) showed that combining nitrogen fertilizer with biochar application enhances plant nitrogen adsorption and assimilation, improving soil fertility and promoting crop growth. This strategy increases nutrient availability, enhances nitrogen uptake, and ultimately boosts crop production. Studies have shown that the free-living *Trichoderma* strains obtained from the rhizosphere exhibited remarkable proficiency in mobilizing insoluble phosphate. Specifically, *T. virens* and *T. koningii* strains displayed substantial effectiveness in solubilizing phosphate (Joo and Hussein, 2022). The application of 3% rice straw biochar along with *T. viride* exhibited a substantial increase in phosphorus content, highlighting the potential synergistic effects of biochar and biocontrol agent application on phosphorus uptake in absence of *M. phaseolina* (Saeed *et al.*, 2023).

We found that the 3% rice straw biochar suppressed the charcoal rot disease in maize. Numerous scientific investigations have suggested that biochar exhibits promising potential as an effective adsorbent and inducer of plant resistance, for mitigating the severity of plant diseases (Mehari *et al.*, 2015). Eo *et al*. (2018) described that the rice husk biochar was efficient in controlling the root rot disease and also promoting the growth of beneficial organisms as well as suppressing the other pathogens (*Cylindrocarpon destructans* and *Fusarium solani*). Frenkel *et al*. (2017) examined the hormesis effect of biochar on plant growth and disease suppression which reported that lower concentrations (≤1%) of biochar tend to exhibit disease-suppressing properties against several diseases. Conversely, higher concentrations (≥3%) were generally found to be ineffective or even capable of inducing plant diseases. Jaiswal *et al*. (2014) revealed that biochar at lower concentration increased the growth of cucumber plants and suppressed the damping off disease caused by *Rhizoctonia solani,* while at higher concentration biochar was totally ineffective in plant growth promotion also induced the disease incidence and severity. The synergistic effects of farmyard manure biochar and PGPR (*Planomicrobium sp.*) along with sulfur demonstrated notable efficacy in the control of charcoal rot disease in sunflower plants. This efficacy is attributed to their collective impact on modulating the activities of antioxidant enzymes and enhancing the immunity of sunflower plants (Ijaz *et al*. 2021). Similarly, rice straw biochar at lower application rates was found to be more effective in controlling charcoal rot disease either with or without *T. viride* in maize under glasshouse conditions. The present findings will be a step forward towards sustainability and reliance on organic approaches of plant protection.

Conclusion and future prospects: Rice straw biochar was effective in controlling *M. phaseolina* in maize. Overall 3% rice straw biochar was effective in promoting maize growth both in the presence or absence of *T. viride.* The spongy structure of biochar supports beneficial biocontrol microbes which act as antagonist to other pathogens. Thus, the amendment of biochar had an impact on soil fertility, plant growth and disease suppression at varying levels dependent upon the concentration of biochar applied as a soil amendment. Further studies should be done to understand the mechanism of biochar utilized in the suppression of charcoal rot in maize and to analyze the efficiency of various biochar types against different pathogenic bacterial and fungi.

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