Effect of Biochar and Seed Biopriming with PGR On Maize Anthracnose

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With global food security being under threat due to crop pathogens, the use of chemical pesticides results in soil degradation, resistant pathogens, and environmental hazards. Biochar acts as an eco-friendly source of managing plant pathogens mitigating disease severity and enhancing soil quality. The objective of this research is to evaluate the synergetic effect of Rice Straw Biochar (RB) with Plant Growth-Promoting Rhizobacteria (PGPR), specifically *Bacillus subtilis* (BS) and *Pseudomonas fluorescens* (PF) against anthracnose in maize caused by *Colletotrichum graminicola* (CG). Inoculated and uninoculated maize were gown in 3% Rice straw biochar under greenhouse conditions, with and without PGPRs. The treated plants demonstrated significant improvements in growth, shoot, and root mass while also enhancing antioxidant enzymatic activities, reducing the disease severity by up to 80%. The synergistic effect of biochar and PGPRs not only suppressed *C. graminicola* growth but also improved soil fertility and plant nutrient uptake. This study has revealed the combined application of biochar with PGPRs enhanced plant growth and soil quality by improving the uptake and absorption of nitrogen by the plant. And provided resistance in maize against *C. graminicola*. Moreover, the plants grown in 3% RB amended soil, treated with PGPRs showed increased activity of anti-oxidant enzymes peroxidase and catalase along with higher chlorophyll contents compared to the non-amended plants proving the integrative strategy to be a sustainable alternative to chemical pesticide, providing an environmentally friendly approach to combat plant pathogens.

Abbreviations:

Rice Straw Biochar (RB)
Plant Growth-Promoting Rhizobacteria (PGPR)
Bacillus subtilis (BS)
Pseudomonas fluorescens (PF)
Colletotrichum graminicola (CG)
Fungal Culture Bank of Pakistan (FCBP)
Faculty of Agriculture Sciences (FAS)
Electric Conductivity (EC)
Colony Forming Units (CFU)
Days After Incubation (DAI)
Lauria-Bertani (LB)
Cation Exchange Capacity (CEC)

Keywords: Rice Straw Biochar, Plant Growth-Promoting Rhizobacteria (PGPR), Anthracnose Disease Management, Maize (Zea mays), Sustainable Agriculture

Introduction:

Farming is a crucial source of food, housing, and fiber. Pakistan's economy is dependent on agriculture, which accounts for 24% of Gross Domestic Product (GDP) and 37.4% of jobs in the nation [1]. Maize (*Zea mays L.*) is a cereal grain belonging to the Gramineae (Poaceae) family [2]. It is originally found in America, is the most widely cultivated edible grain crop worldwide [3]. It is used as a vital food source as well as for animal feed and can be used commercially as raw material for various industries and as a source of biofuel [4]. In comparison to other cereal grains, maize as a feed may be quickly converted into meat, milk, and other products. Due to being high in net calories and low in fiber and protein, maize acts as a good feed grain [5].

Pests and crop diseases are seriously threatening corn productivity and quality [6]. *Colletotrichum graminicola* is among the most common and economically important maize diseases, resulting in anthracnose stalk rot and leaf blight [7]. The disease infects all plant parts, however it mostly damages the leaves and stem [8]. During severe infections, maize leaves may experience reduced photosynthetic area, resulting in early leaf senescence. During the early stages of grain production, the upper foliage and stem internodes die prematurely, causing top dieback symptoms and a drop in yields [9].

Biochar is a type of charcoal created from biomass and incorporated into soil [10]. Previous studies prove that biochar is involved in improving soil fertility while also reducing climate change through carbon sequestration [11]. Biochar is a solid carbon-rich product that results from biomass pyrolysis and has recently been recognized for its numerous agricultural benefits. It is composed of carbon, hydrogen, potassium, nitrogen, and magnesium, all of which are required by plants for their development [12]. Biochar increases soil physicochemical and biological properties by incorporating more organic matter [13]. However, the overdose of biochar affects plant growth by supporting plant pathogens and providing a feasible environment for them to grow and infect the crop [14]. Rice husk is prepared during the initial rice milling state when paddy rice is hulled. Similar to other plant waste, rice husk can be transformed into biochar. Rice husk and straw-derived biochar are reported to improve soil alkalinity better than other biochar sources because they possess higher cation exchange capacity and available phosphorus [15]. Rice Biochar (RB) components include approximately 20% of the rice's weight and contains, 50% cellulose, 15%-20% silica, 25%-30% lignin, and 10%-15% moisture [16].

Upon being infused into seeds, Plant Growth-Promoting Rhizobacterium (PGPR) infiltrates the roots and accelerates plant growth. PGPRs positively impact crop health by producing phytohormones, degrading soil organic matter, depleting agricultural residues, and suppressing plant pathogens [17]. Bacillus subtilis is a widely distributed bacterium that secretes a range of compounds of biological significance and endospores, rendering it a desirable biocontrol agent. Similarly, Pseudomonas fluorescens, due to its strong ability to occupy natural rhizospheres, is a potential biocontrol bacterium. Its widespread presence along with unique traits contribute to its role as a plant promoting bacteria [18].

Objectives and Novelty:

Seed priming with PGPR is a modern approach to disease management of plants. Various crops including peas, maize, safflower, tomatoes, and brinjal have shown enhanced germination, vigor, viability, and growth in response to seed biopriming [16]. Corn seed biopriming with this technique (Bacillus spp. and Pseudomonas spp.) increases plant fresh weight. When applied in combination with PGPR, biochar enhances soil texture, and structure, reduces salt stress, and contributes towards plant nutritional balance [19].

There is a need for modern disease management techniques that encompass reduced environmental pollution caused by the use of pesticides and enhanced crop protection against pathogenic damage. Keeping this need in view, a current study has been carried out to analyze

the interactive effect of growth promoting bacteria for plants along with rice straw biochar on plant development, nutritional balance along resistance against anthracnose in maize.

Material and Methods:

Figure 1 provides the flow chart of the methodology adopted to evaluate the effect of biochar on the maize anthracnose along with PGPR.

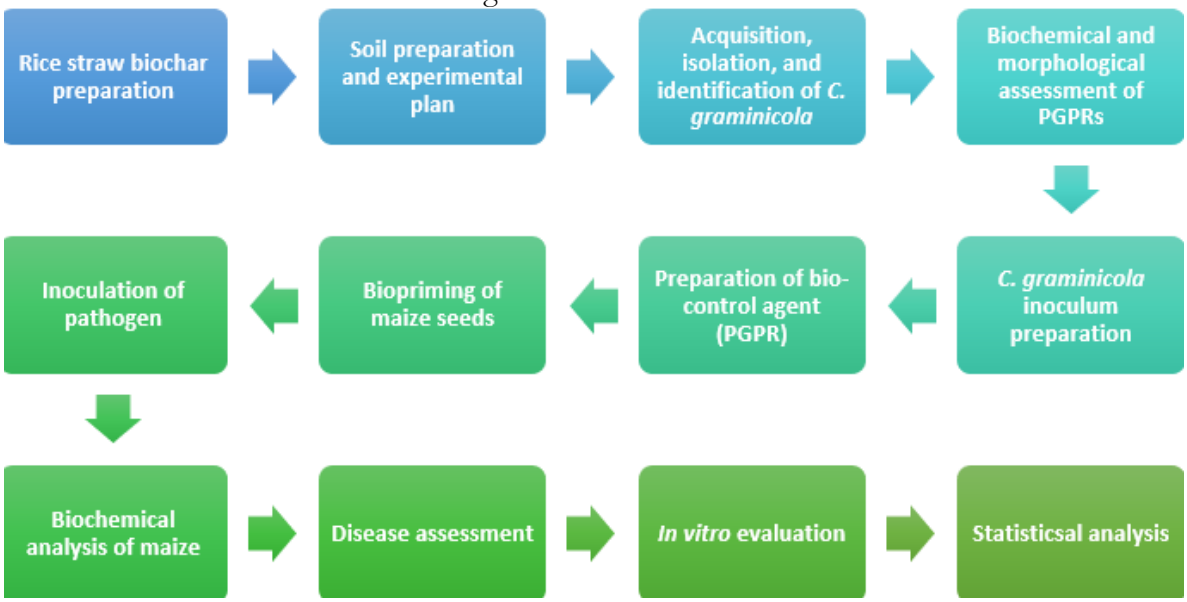

Figure 1. Flow diagram of research methodology.

Rice Straw Biochar Preparation:

Rice straw biochar was prepared by pyrolysis of straws of rice in a portable kiln at 400. The primary burner with holes created at the base was filled with rice straw, and the airflow was controlled by the chimney and adapter. The biochar was sprayed with tap water post-pyrolysis which reduced the temperature, resulting in sieved powder biochar [1]. Biochar properties were analyzed as per protocols mentioned by [20] for pH and Electric Conductivity (EC), while carbon and nitrogen contents of biochar were assessed by a CNS analyzer [21]. Biochar prepared had a pH of 8.4, EC of 2.9 dsm^{-1} determined by following the protocol, carbon and nitrogen content of 42% and 6g kg^{1}, respectively.

Soil Preparation and Experimental Plan:

In this study, Formalin-sterilized sandy loam soil was utilized which was later classified as sandy loam soil consisting of 19% clay particles, 2% silt particles, and 79% sand particles. The sterilized soil was then mixed with 3% (v/v) rice straw biochar and 10% of organic compost. The following treatment plan was implemented for the experiment to cultivate maize plants: (a) soil only, (b) soil amended with 3% rice-straw biochar (RB3%). The plants were either treated with *Bacillus subtilis* (+BS) or *Pseudomonas florescence* (+PF) as biocontrol agents or remained untreated (-BS) or (-PF) and inoculated with *Colletotrichum graminicola* (+CG) or left uninoculated. Each treatment was allocated 5 replicates in a fully randomized design. The plants were maintained at 30°C, with a relative humidity of approx. 50%.

Acquisition, Isolation, and Identification of Colletotrichum Graminicola:

The biocontrol agents *B. subtilis* and *P. fluorescens* slants and the pure culture plate of *C. graminicola* were acquired from the Fungal Culture Bank of Pakistan (FCBP), Faculty of Agriculture Sciences (FAS), University of the Punjab, Lahore. Both macroscopically and microscopically, the morphology of *C. graminicola* was investigated. Macroscopic characteristics were indicated by colony color, growth rate, and texture. Microscopic aspects such as hyphal properties, the conidial body, appressoria formation, and other structures were examined using a compound microscope with 400x magnification. The fungal mycelia were observed on a glass

slide with a lactophenol blue stain used for visualization. The ingredients were carefully mixed and observed under a microscope.

Biochemical and Morphological Assessment of PGPRs:

Isolates of *B. subtilis* and *P. fluorescens* were identified through colony morphology, growth pattern, and biochemical traits including gram staining procedure, where a smear of bacterial culture was prepared, heat fixed, a couple of drops of crystal violet were put on smear for 1 minute. It was washed with running tap water and iodine was flooded to smear and let it sit for 2 minutes. The stain was decolorized by drop-wise application of 95% ethyl alcohol. After that, a few drops of safranine were poured over it. The slide was washed under running tap water and observed under a microscope. For catalase production, a drop of 30% H_2O_2 was applied over the test culture on a clean glass slide and results were reported in the form of bubble production.

Graminicola Inoculum Preparation:

The inoculum suspension was produced from a two-week-old *C. graminicola* culture inoculated on Potato Dextrose Agar (PDA) medium. To prepare the inoculum suspension, mycelial colonies were taken from culture plates and suspended in the mixture of deionized sterile water and 0.02% Tween 80. The colony's surface was scraped with the help of a glass slide. The solution was then taken from the top of the plate with a pipette, filtered through filter paper, and put into a conical flask. To separate and settle conidia, the solution was centrifuged at 10,000 rpm for 5 mins. The final conidial count was adjusted to 1.0×10^6 conidia/ml using a hemocytometer.

Preparation of Bio-Control Agent (PGPR):

The inoculum of *Bacillus subtillis* and *Pseudomonas fluorescence* was cultured on Lauria-Bertani (LB) broth. Inoculated suspensions were placed on a shaking incubator at 100 rpm and 25-300C. Using a spectrophotometer, by adjusting optical density at 0.8 and wavelength at 600 nm, the final conidial count was adjusted to 1.0×10^6 conidia/ml.

Biopriming of Maize Seeds:

Surface sterilization of maze seeds was achieved by soaking the seed in 0.2% solution of NaOCl for 3 minutes and, afterward, cleaning with distilled water. Sterilized seeds were soaked in a bacterial suspension of about $1.0x10^8$ Colony Forming Units (CFU) /ml for 16 hours at 37°C and air dried. These seeds were sown in pots at a depth of 3 cm.

Inoculation of Pathogen:

Three days after seedling emergence, a spore suspension (1.5 cc) of *C. graminicola* was injected into the middle of the maize stalk using a sterilized syringe at an angle.

Assessment of Maize Growth:

Maize growth was measured by determining root fresh weight, shoot fresh weight, and root and shoot length 70 days post-inoculation. Samples were dried at 38^0C for 14 days to determine dry root and shoot weight. Each treatment further contained 5 samples and the readings were averaged for every treatment.

Biochemical Analysis of Maize:

The total chlorophyll content of plant leaf extract was measured by determining the content of chlorophyll A and B at wavelengths of 663 and 645 nm respectively by using a spectrophotometer. Total chlorophyll content (mg/g fresh leaf weight) was measured by putting spectrophotometer readings for chlorophyll a and b in the following formulae.

$$Total\ chlorophyll\ contents = chlorophyll\ \mathrm{a} + chlorophyll\ \mathrm{b}$$

$$Chlorophyll\ a = \frac{12.7(A663nm) - 2.69(A645nm)}{1000}(mg/g)$$

$$Chlorophyll\ \mathrm{b} = \frac{22.9(A645nm) - 4.68(A663nm)}{1000}(mg/g)$$

Catalase and Peroxidase concentration was measured using a visible spectrophotometer. Researcher employed the guaiacol technique to measure peroxidase activity and the UV absorption method to measure catalase.

Disease Assessment:

Anthracnose disease on maize leaves and stems was visually assessed three weeks after pathogen inoculation based on disease signs appearing on leaves. A disease rating scale which ranged from 0 to 5 was used to evaluate the results of the treatment on disease severity (Table 1).

Table 1: Disease Severity Rating Scale (0-5) for Anthracnose Assessment.

Rating	Disease Intensity %	Level
0	0%	Immune
1	*1-10%*	*Resistant*
2	11-25%	Moderately resistant
3	26-49%	Moderately susceptible
4	50-74%	Susceptible
5	75 and above	Completely susceptible

In vitro Evaluation of Biochar, Bacillus Subtilis, and Pseudomonas Fluorescens Against *Colletotrichum graminicola*:

To assess the antagonistic activity against the fungal pathogen *C. graminicola*, rice straw biochar and five-day-old cultures of *B. subtilis* and *P. fluorescens* were tested in vitro on PDA plates (Table 2). Before being put into PDA media plates, rice straw biochar was sieved via a 100 µm sieve [22]. To create a this medium, a sterile 250 ml flask containing 3% (v/v) rice straw biochar was used. After autoclaving, the medium was transferred to Petri dishes with a diameter of 90 mm and allowed to set. Agar plugs of 5 mm were placed at the center of the petri plate, and taken from the actively growing parts of the fungal cultures.

Furthermore, both bacterial strains were put on agar plates at the petri plate borders, and the antifungal effect of the PGPR strains was assessed using a dual culture approach. Using the *C. graminicola* infected disc, *B. subtilis* and *P. fluorescens* were inoculated on the petri dish containing PDA where biochar was used in three distinct locations around the Petri plate. In the control treatment, instead of biochar and PGPR *C. graminicola* was inoculated. The fungus was incubated at 25 ± 3°C, and the growth inhibition of *C. graminicola* was assessed at the interval of 3, 5, and 7 Days After Incubation (DAI). Seven treatments were designed using the dual culture method, with each treatment replicated three times. Fungal colony diameter was measured, after which the percentage of inhibition was calculated by the method outlined below.

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

Where C is the colony diameter (cm) of the plates of a control group and T is the colony diameter in cm of the plates of the treated group.

Statistics Analysis:

Data for each analysis were provided as mean ± SD from five replicates. Statistics 8.1 was utilized to carry out the statistical analysis. The data was studied using One-way ANOVA (Analysis of variance), and for mean comparisons, Tukey's HSD test ($P < 0.05$) was applied.

Table 2: Experimental Plan for In vitro Fungal Growth Inhibition Assay

Treatment	Description
T1	Colletotrichum graminicola
T2	Bacillus subtilis + Colletotrichum graminicola
T3	Bacillus subtilis + Colletotrichum graminicola + Rice straw Biochar
T4	Pseudomonas fluorescens + Colletotrichum graminicola
T5	Pseudomonas fluorescens + Colletotrichum graminicola + Rice straw Biochar
T6	Bacillus subtilis + Pseudomonas fluorescens + Colletotrichum graminicola +
T7	Rice straw Biochar + Colletotrichum graminicola

Results:

Comparative Analysis of In Vitro Efficiency of Biochar and PGPRs against the Pathogen:

In the absence of biochar, *B. subtilis* (BS+CG–RB) and *P. fluorescens* (PF+CG–RB) exhibited moderate antagonistic activity, inhibiting radial growth by 46.95% and 37.63%, respectively, as compared to the control (Figure 2). However, combined treatment of *P. fluorescens* and rice straw biochar (RB+PF+CG) significantly improved the inhibitory effect, reducing radial growth by 53.04%. Similarly, the combination of *B. subtilis* with rice straw biochar (BS+CG+RB) enhanced inhibition to 58.78%. The most potent treatment was the combination of both PGPR strains with rice straw biochar (BS+PF+CG+RB), which reduced the radial growth of *C. graminicola* by 71.68%. In comparison, the treatment with rice straw biochar alone (without PGPR strains) inhibited the pathogen by 50.17% (Table 3). These findings highlight the combined effect of biochar and PGPR strains in suppressing *C. graminicola*.

Table 3: Growth inhibition of *C. graminicola* in invitro analysis using PGPRs and biochar.

Treatment	Mean Radial Growth (cm)±SD	Growth Inhibition percentage %
CG	2.79±1.50	-
CG+BS	1.48±0.89	46.95^{b}
CG+BS +RB	1.15±0.68	58.78^{bc}
CG+PF	1.74±0.86	37.63^{ab}
CG+PF+RB	1.31±0.83	53.04^{b}
CG+BS+PF	0.79 ±0.75	71.68^{c}
CG+RB	1.39±0.80	50.17^{a}

Tukey's HSD test ($P < 0.05$) shows a significant variation in values, denoted by distinct superscripts, while SD represents standard deviation.

Shoot Length of Maize:

The treatment with *B. subtilis* and rice straw charcoal (Biochar+BS) without *C. graminicola* resulted in the longest shoot length (78 cm) (Table 4). Similarly, plants infected just with *C. graminicola* (Soil+CG) had the shortest shoot length (41.7 cm), showing the pathogen's impact on plant development (Figure 3). Under pathogen stress conditions, the combination of rice straw biochar and PGPRs greatly increased shoot length. Treatments like Biochar+BS+CG and Biochar+PF+CG enhanced shoot length by 67.8% and 71.6%, respectively, in comparison to the infected control (S+CG). These findings demonstrate the benefits of PGPR seed priming and rice straw biochar in minimizing the deleterious influence of *C. graminicola* on maize development (Figure 4).

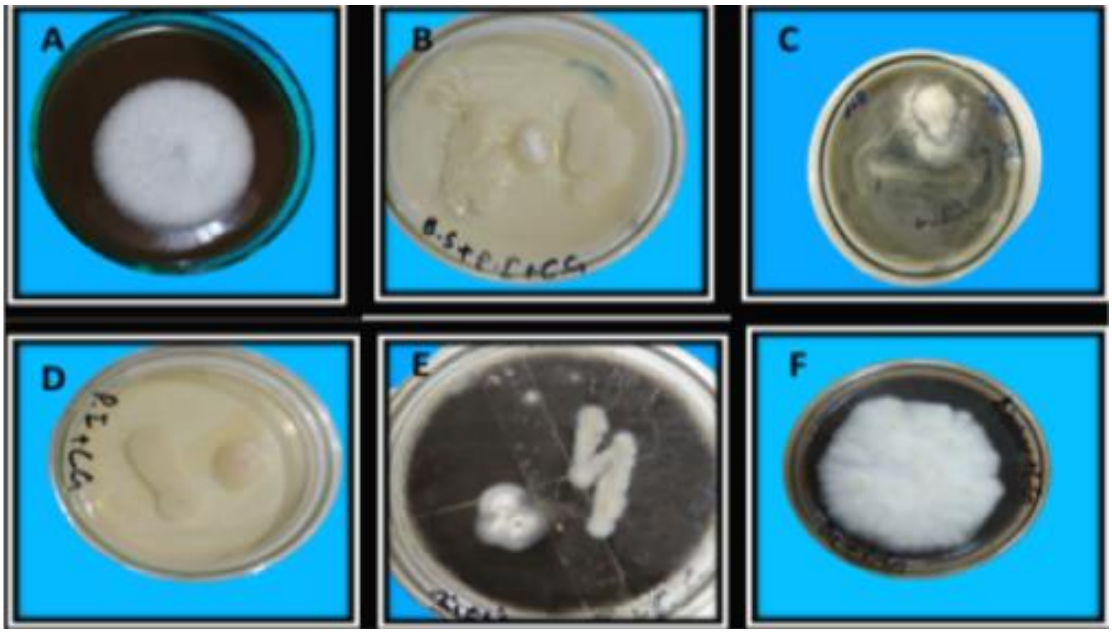

Figure 2: The effect of PGPRs (BS and PF) on the fungal mycelial growth of pathogen (CG) with and without the use of biochar. Pathogen alone (control), Pathogen with (A); BS+PF(B); PF+RB (C); PF(D); BS+RB (E); and RB (F).

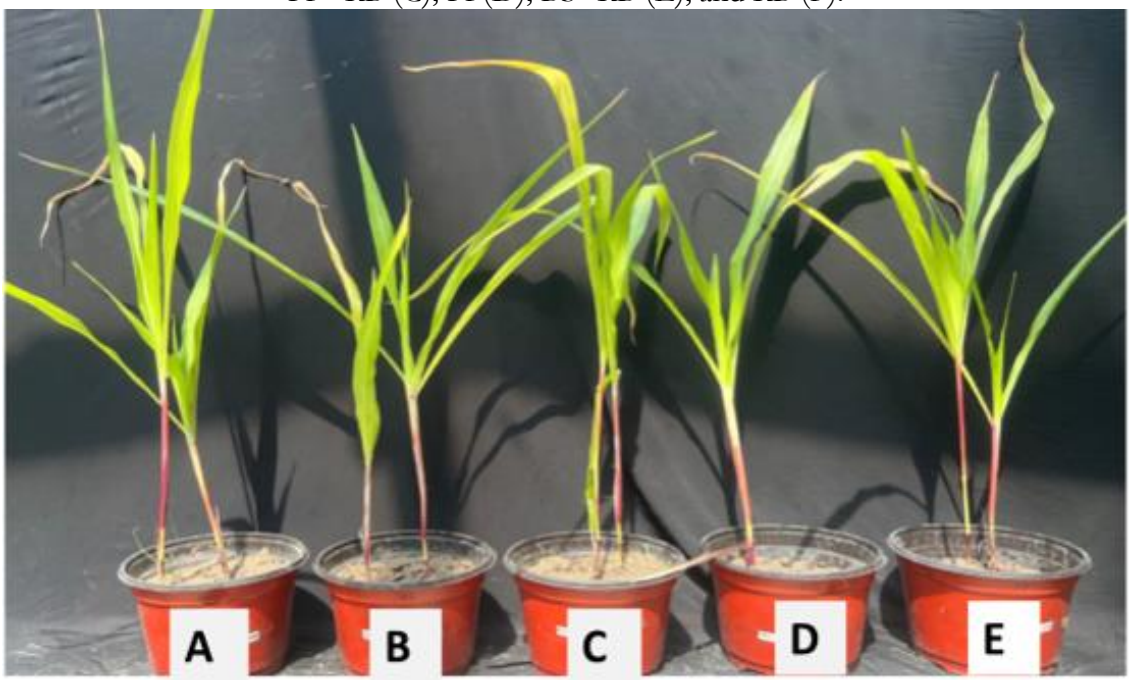

Figure 3: Effect of RB on shoot height of maize plants. Treatments include: (A) Unamended 3%RB-CG, (B) Amended +CG, (C) Uninoculated S+BS-CG, (D) Inoculated S+BS+CG, and (E) 3% RB Uninoculated.

Figure 4: Effect of RB and *P. fluorescens* on maize shoot length in inoculated plants (H, J) or uninoculated plants (G, I). Treatments include: (F, G) 3% RB Unamended and Uninoculated, (H) 3% RB Inoculated, (I) PF+RB Uninoculated, and (J) PF+RB Inoculated.

Root Length of Maize:

Significant improvements were observed in treatments combining rice straw biochar and PGPRs. The highest root lengths were recorded in treatments with 3% rice straw biochar combined with *P. fluorescens* (Biochar+PF, 35.0 cm) and rice straw biochar alone (33.1 cm). In contrast, maize plants treated with *C. graminicola* in the control group (S+CG) exhibited the shortest length of root which was 5.2 cm, indicating the pathogen's impact on root growth (Figure 5). These results indicate that rice biochar (3%) and seed treated with *B. subtilis* as well as *P. fluorescens*, significantly enhance root mass in maize, while simultaneously negating the negative effects of infection caused by *C. graminicola* (Table 4).

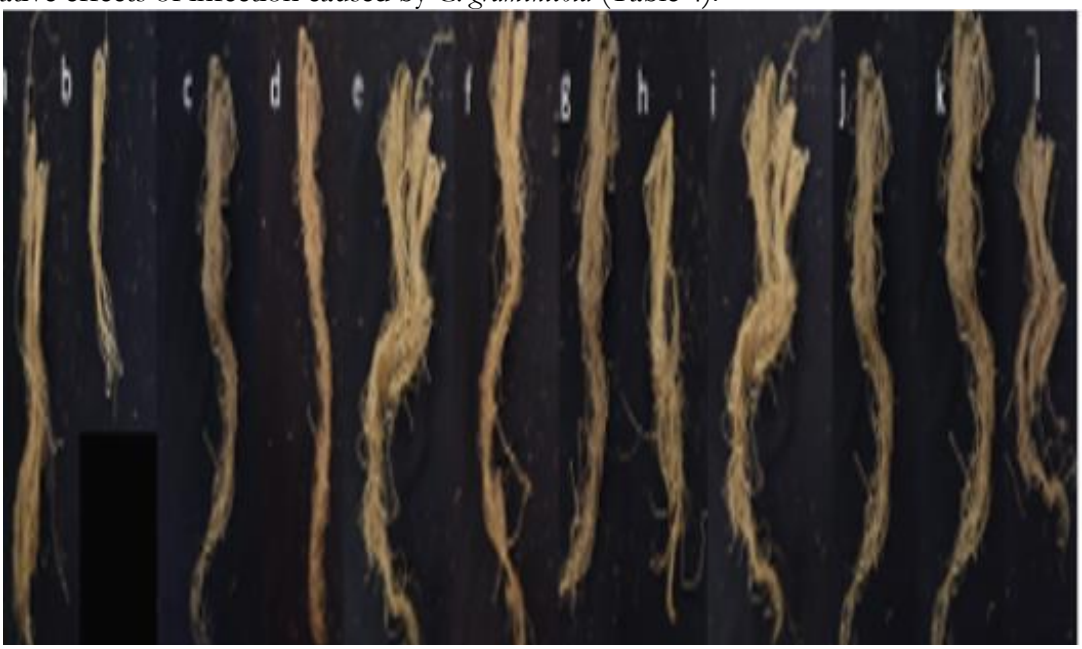

Figure 5: The effect of RB and PGPRs (BS and PF) on maize root growth in inoculated and uninoculated plants. Treatments: (a) Control (Soil Alone); (b) Soil + CG; (c) Soil + BS + CG; (d) Soil + BS + CG; (e) 3% RB Uninoculated; (f) 3% RB + CG; (g) Soil + PF Uninoculated; (h) Soil + PF Inoculated; (i) 3% RB + PF Uninoculated; (j) 3% RB + PF Inoculated; (k) 3% RB + BS + CG; (l) 3% RB + BS + Inoculated.

Table 4: Effect of biochar, PGPR, and pathogen on the length parameters of plants.

Sr.	Treatment	Shoot length (cm)±SD	Root length (cm)±SD
1	Soil	58.4 ± 2.56^{bc}	14.8 ± 1.4^{c}
2	BS	68.7 ± 2.51^{ab}	32.1 ± 4.04^{a}
3	Soil+CG	41.7 ± 3.04^{c}	5.1 ± 1.92^{d}
4	BS+CG	67.8 ± 5.2^{ab}	25.6 ± 1.81^{ab}
5	Biochar	70.2 ± 4.32^{ab}	34.0 ± 2.57^{a}
6	Biochar+CG	59.7 ± 9.48^{abc}	20.1 ± 1.93^{bc}
7	Biochar+BS	78.0 ± 6.12^{a}	32.4 ± 5.26^{a}
8	Biochar+BS+CG	67.8 ± 10.9^{ab}	26.4 ± 2.07^{ab}
9	PF	74.1 ± 14.20^{ab}	27.3 ± 1.93^{ab}
10	PF+CG	65.9 ± 12.8^{ab}	31.1 ± 3.37^{ab}
11	Biochar+PF	71.2 ± 12.0^{ab}	35.0 ± 2.39^{ab}
12	Biochar+PF +CG	71.6 ± 4.85^{ab}	19.8 ± 3.11^{a}

Tukey's HSD test ($P < 0.05$) shows a significant variation in values, denoted by distinct superscripts, while SD represents standard deviation.

Dry Shoot Weight of Maize:

The dry weight of maize stems was evaluated by dehydrating the plant material at 38°C for 14 days. The highest dry shoot biomass was recorded in treatments that combine biochar (3%) and PGPRs, both plants inoculated and uninoculated with *C. graminicola* (Figure 6). Similarly, the lowest value of dry shoot weight (1.36 g) was seen in the control treatments (S+CG), in the presence of *C. graminicola* but neither seed priming nor biochar was applied. These results demonstrated the positive impact of biochar and PGPRs on improving shoot weight under disease conditions.

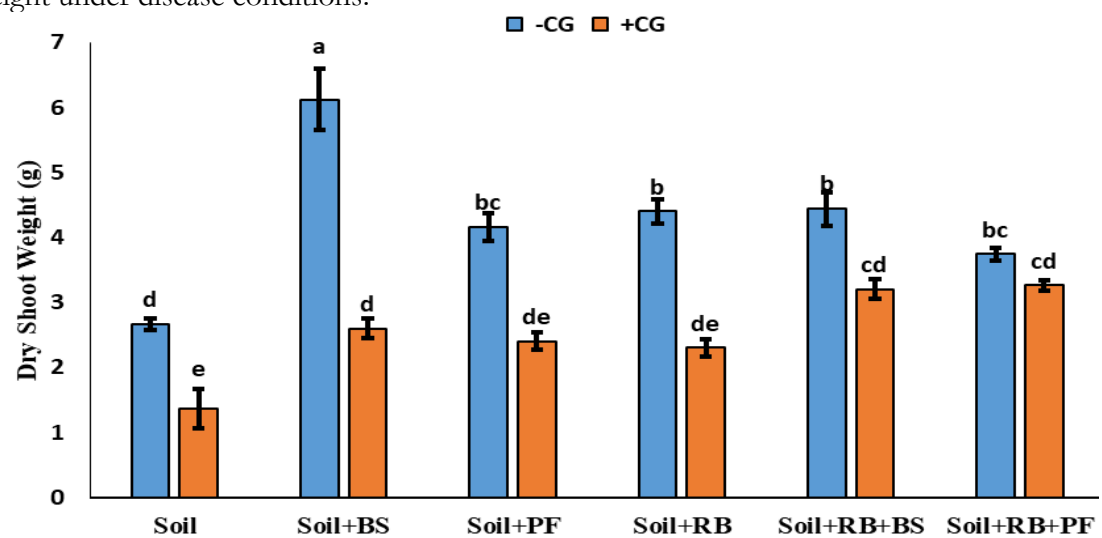

Figure 6: Effect of biochar, PGPRs, and *C. graminicola* on maize dry shoot weight. Conditions include: (+CG) or (-CG) for *C. graminicola*, (+BS) or (-BS) for *B. subtilis*, and (+PF) or (-PF) for P. fluorescens. Tukey's HSD test ($P < 0.05$) highlights significant differences, represented by symbols above the error bars.

Dry Root weight of Maize:

The dry root weight was determined by dehydrating the roots at 38°C for 14 days. The results indicated that treatments with 3% rice straw biochar combined with PGPR biopriming, either in the presence or absence of *C. graminicola*, produced the highest dry root biomass values. The maximum dry root biomass (2.34 g) was recorded in the treatment with 3% biochar alone (S+3%RB, -CG), without pathogen or PGPR application. However, the control (S+CG)

exhibited the lowest value (0.6g), where plants were inoculated with *C. graminicola* alone and did not receive biochar or PGPRs (Figure 7).

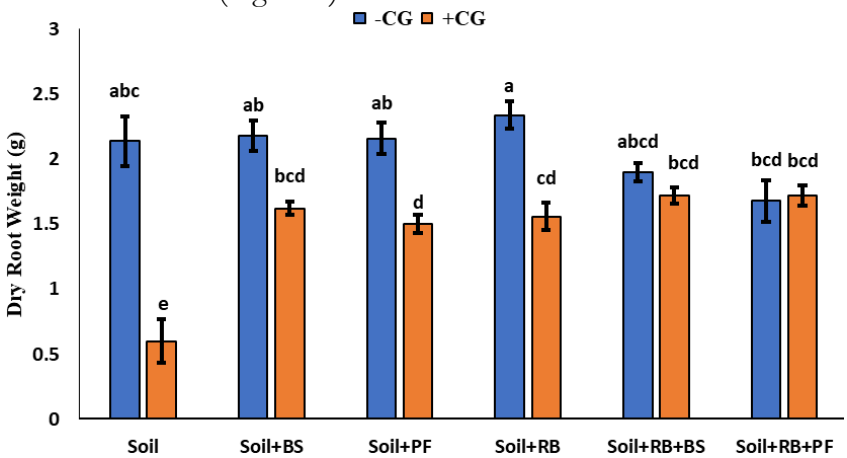

Figure 7: The effect of biochar, PGPRs, and *C. graminicola* on maize plant dry root weight. Conditions include: (+CG) or (-CG) for *C. graminicola*, (+BS) or (-BS) for *B. subtilis*, and (+PF) or (-PF) for *P. fluorescens*. Tukey's HSD test ($P < 0.05$) indicates significant variations, denoted by symbols above the columns.

Enzymatic Antioxidant Activity:

The biochemical activity of antioxidants, specifically peroxidase and catalase, was significantly influenced by the application of biochar (3%) along with PGPR seed treatment with *B. subtilis* and *P. fluorescens* ($P < 0.05$; (Table 5). Treatments combining rice straw biochar (3%) and PGPRs under pathogen stress demonstrated the highest enzymatic activities. The peroxidase activity reached 4.39 and 4.53 units/mg, while catalase activity was recorded at 1.35 and 1.26 units/mg, respectively. In treatments without biochar (BS+CG and PF+CG), plants inoculated with *C. graminicola* also showed elevated antioxidant enzyme activity. The POD activity was 3.38 and 3.41 units/mg, respectively, highlighting the role of PGPRs in enhancing antioxidant defense mechanisms. These observations indicated that the collective application of rice straw biochar and PGPRs significantly boosts the activity of enzymatic antioxidants, playing a critical role in inducing resistance against *C. graminicola.*

Chlorophyll Concentration in Maize:

Variations in total chlorophyll contents of maize plants in response to treatment with rice straw biochar, seed biopriming by using *Bacillus subtilis* and *Pseudomonas fluorescens* along stress caused by *C. graminicola* was determined by measuring chlorophyll a and chlorophyll b concentrations at 645 nm and 663 nm, respectively. The results indicated no significant differences in the total chlorophyll content across the treatments, regardless of the application of rice straw biochar, PGPRs, or pathogen inoculation (Table 5). This suggests that while the treatments had a significant impact on other growth and defense parameters, the chlorophyll content of maize plants remained relatively unaffected.

Table 5: Impact of Biochar, PGPRs, and *C. graminicola* on Enzymatic Activity (POD, COD) and Total Chlorophyll contents in Maize.

Treatment	Peroxidase	Catalase	Chlorophyll
Soil	3.01 ± 0.13^{f}	0.34 ± 0.04^{f}	0.30
BS	3.11 ± 0.10^{ef}	0.370 ± 0.03^{f}	0.26
Soil+CG	3.13 ± 0.13^{def}	0.63 ± 0.05^{cd}	0.16
BS+CG	3.38 ± 0.04^{cde}	0.37 ± 0.05^{c}	0.04
Biochar	3.38 ± 0.08^{cde}	0.47 ± 0.03^{ef}	0.11
Biochar+CG	4.12 ± 0.13^{b}	0.93 ± 0.06^{b}	0.12
Biochar+BS	3.38 ± 0.07^{cde}	0.55 ± 0.04^{de}	0.12

Biochar+BS+CG	4.53±0.04[a]	1.35±0.05[a]	0.15
PF	3.20±0.60[cdef]	0.47±0.03[ef]	0.16
PF+CG	3.41±0.03[cd]	0.58±0.05[de]	0.02
Biochar+PF	3.42±0.13[c]	0.69±0.05[cd]	0.10
Biochar+PF+CG	4.39±0.10[ab]	1.26±0.08[a]	0.05

Tukey's HSD test ($P < 0.05$) shows a significant variation in values, denoted by distinct superscripts, where SD represents standard deviation.

Disease Assessment:

The impact of *C. graminicola* on maize plants was evaluated during the tasseling stage. The results revealed that the combination of 3% rice straw biochar and *P. fluorescens* (Biochar+PF+CG) effectively suppressed the disease, showing resistance against the pathogen with an incidence of 40% and a severity of 20%.

In comparison, the maximum incidence (100%) and severity (75%) were observed in the untreated control plants (Soil+CG), which were inoculated with *C. graminicola* without any biochar or PGPR application. Plants treated with Bacillus subtilis and 3% rice straw biochar (Biochar+BS+CG) showed a moderate reaction in response to disease, where disease incidence was 60% and severity was 40%, indicating a mildly susceptible reaction (Table 6).

Table 6: Impact of biochar and PGPRs on the disease development in maize.

Treatments	Disease Incidence	% Severity Index	Plant Response
Soil+CG	80[b]	62[c]	Susceptible
Soil+BS+CG	100[a]	75[a]	completely susceptible
Biochar+BS+CG	60[c]	40[e]	Moderately susceptible
Soil+Biochar+CG	60[c]	50[d]	Susceptible
Soil+PF+CG	80[b]	70[b]	Susceptible
Biochar+PF+CG	40[d]	20[f]	Moderately susceptible

Discussion:

Plant diseases and pathogens are prevalent in agricultural food production. Insects and weeds result in 20-40% of yield losses worldwide [23]. *C. graminicola* is one of the most virulent seed-borne fungal plant pathogens responsible for maize anthracnose. During maize harvest, anthracnose results in significant yield loss and causes lodging, with it prevailing due to changing climatic conditions. [24]. It lives in seed, making it difficult to control with traditional pesticides. Integrated disease management approaches that include biocontrol agents, soil amendments, and resistant varieties should be employed for effective disease management. For more than 2 decades, compost and biochar for upregulating crop yield and mitigating disease stress.

Research work is being done on biochar as a source of carbon sequestration with a primary goal of enhancing agricultural output, and improving soil fertility and water-holding capacity by using biochar [25]. A current study was conducted to develop a sustainable and economically efficient approach to minimize agricultural yield loss due to plant diseases and preservation of biodiversity. Previous research revealed the beneficial impact of compost for decreasing anthracnose incidence in maize [26]. It has been confirmed by many researchers that soil amendment with compost lowers disease severity due to soil or seed-borne pathogens by interfering with soil microbial dynamics. However, there are presently several studies available on the effect of biochar on maize anthracnose.

Chemical pesticides are more effective in controlling seed-borne fungal pathogens but are more expensive and hazardous to the environment. Many studies have employed PGPR as a biocontrol agents to tackle anthracnose disease associated with maize. This approach shows significant potential in improving plant health as well as reducing the need for chemical

pesticides proving an effective biological control method [27]. Thus, using diverse experimental methodologies, biological control has recently been implemented on an industrial scale. These bacteria live in close vicinity to lower plant parts. They either boost the development of the plant in the absence of a pathogen or can indirectly negate the harmful effect of phytopathogens through mechanisms like ISR, antibiosis, HCN production, competition, and siderophore production. [28].

To reduce *C. graminicola* growth in maize, this study employed PGPR bacteria *Bacillus subtilis, Pseudomonas fluorescens*, and rice straw biochar as soil amendments for the first time. Comparative research on biochar along with seed priming with these amendments proved that application of biochar, compost, and PGPR all enhanced soil nutritional status, water holding capacity, and Cation Exchange Capacity (CEC), while also reducing carbon dioxide emissions in the soil. This data showed that the usage of these soil amendments affected plant development, nutritional levels, frequency range, biological responses along the degree of disease. It was noticed that using rice straw biochar increased the antioxidant enzymes peroxidase and catalase, as well as the chlorophyll in maize shoots.

To evaluate the effect of bacterial isolates on diseases, in vitro analysis is performed followed by greenhouse and field trials [29]. The examination of performance consistency is often conducted across many geographic locations and climatic conditions. Crop species are also considered since rhizobacteria have close associations with their host plants [30]. To evaluate the existence of PGPR and their interaction with their host plants their growth in the rhizosphere can be tracked. Their growth can be easily monitored in greenhouse conditions due to the presence of constant environmental conditions in the glasshouse.

Abiotic stress might now be taken into account when examining performance under various climate change scenarios. Biochar is regarded to be the most cost-effective way of sequestering carbon in resistant agricultural soils [12]. This study also used biochar to suppress the northern corn leaf blight disease and increase corn production. Based on the results, biochar enhances disease resistance by influencing the competitive environment between seed-borne pathogens and PGPRs. However, higher concentrations of biochar were evaluated to have increased pathogen multiplication in the case of anthracnose. Collective application of PGPR and rice straw biochar (3%) significantly improved plant vigor by suppressing disease stress found that biochar boosted plant vigor and minimized disease stress at lower concentrations (≥3%). Efforts to minimize maize anthracnose whose causal pathogen in C. graminicola results in enhanced growth and higher plant yield [31].

Amending soil with biochar and priming seeds with PGPR induced systemic resistance to an abundance of plant diseases. These diseases include infections caused by *Fusarium oxysporum f. sp. lycopersici* in tomato fruit and *Rhizoctonia solani* infections in cucumbers [32]. Previous studies exhibited combining biochar with plant growth regulating bacteria improved soil color, increased solar radiation absorption and maintained soil surface temperature. According to studies, biochar created from rice straw improves soil fertility and nitrogen retention, increasing cotton yield while decreasing soil nutritional status. Biochar enhances the fertility and structure of the soil. These variations led to a substantial boost in the growth of plants [33]. Earlier research showed that the coactive impact of biochar generated from manure and seed treatment with PGPR boosted maize yield, and biochemical, and physiological characteristics by preventing *C. graminicola* growth [34]. It is observed that the treatments in which biochar was added with PGPRs under the pathogen-inoculated conditions showed the highest levels of peroxidase and catalase activity. Parallelly, these are the same treatments that showed least susceptibility out of all the treatments. These findings align with the fact that increased catalase and peroxidase activity promotes Reactive Oxygen Species (ROS) detoxification, augmenting the plant defense mechanisms and particularly, systemic resistance (ISR and SAR) [35][22]. Soil that had not been treated with *C. graminicola* had the lowest

chlorophyll concentration in maize plants. According to, *C. graminicola* reduced chlorophyll concentrations in maize by 60% in comparison with the control group in a pot study.

Our findings demonstrated that maize plants infected with 3% rice straw biochar and PGPRs showed the greatest increase in growth metrics when compared to those without soil amendments. Plant growth parameters had minimum value in plants treated with pathogen only without any soil amendment or priming with plant growth regulating bacteria. Plant infection with *C. graminicola* has been shown to cause significant damage to the vascular and root systems, potentially interfering with nutrition absorption. According to [20], using biochar in combination with seed biopriming enhances the ability of plants to uptake N_2, leading to a boost in soil fertility and up-regulates crop development. This approach enhances N_2 absorption, availability of nutrient elements, and crop yield. PGPR can convert insoluble phosphorus to soluble form thus enhancing the availability of phosphorus to plants.

When bio-primed seeds were sown in soil amended with 3% biochar, root, shoot lengths, and weights were increased to a significant level. This shows the synergistic effect of biochar and PGPR against maize anthracnose caused by *C. graminicola*. The synergistic effect of biochar and PGPR also resulted in enhanced seed germination and biomass in maize.

Conclusion:

When accompanied by seed priming with PGPR, biochar can upregulate the maize plant growth while mitigating the effect of maize anthracnose caused by *C. graminicola*, Biochar improves the physical and chemical characteristics of the soil. When used instead of traditional disease management methods, biochar reduces the spread of soil-borne plant pathogens. To reduce the negative effects of chemical fertilizers and pesticides, it is necessary to understand the mechanisms of action of PGPR, and biochar and utilize them to stimulate plant defense systems against diseases. This will reduce the negative impacts of plant pathogens while boosting plant growth. Future research should aim at investigating the mechanisms and biochemical modifications brought about by PGPR and biochar against maize anthracnose caused by C. graminicola. We anticipate_increased_demand from both industry and farmers, along with a deeper understanding of how biochar amendments and PGPRs influence plant physiological traits, adaptive responses, signaling, and interactions with other microbes.

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