Sulfur Application Combined with Planomicrobium sp. Strain MSSA-10 and Farmyard Manure Biochar Helps in the Management of Charcoal Rot Disease in Sunflower (Helianthus annuus L.)

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Abstract: Sunflower (Helianthus annuus L.), a member of the Asteraceae, is one of the major oilseed crops around the world. Charcoal rot caused by Macrophomina phaseolina (Tassi) Goid is the most damaging disease of sunflowers globally. Fungicides are mostly used to control charcoal rot; however, these cause environmental pollution and pose adverse effects on the ecosystem. Therefore, ecofriendly management options are inevitable for the management of charcoal rot disease. Plant mineral nutrition, the use of plant growth-promoting rhizobacteria and biochar have recently been manipulated for the management of different plant diseases. However, the interactive effects of all these treatments have rarely been tested on charcoal rot suppression in sunflowers. This study assessed the influence of sulfur (0 and 2.25 mg/kg) combined with farmyard manure biochar (2%), NPK (20:20:20 mg/kg) and three different plant growth-promoting rhizobacteria (PGPR) strains on the charcoal rot suppression growth, yield, biochemistry and physiology of sunflower. The PGPR strains included in the study were Bacillus sp. strain MR-1/2 (regarded as PGPR1), Achromobacter sp. strain FB-14 (regarded as PGPR2) and Planomicrobium sp. strain MSSA-10 (regarded as PGPR3). The charcoal rot infestation was induced by inoculating the soil with Macrophomina phaseolina, and the impacts of the different treatments were studied on the disease infestation, growth, yield, biochemistry and physiology of sunflowers under 0 and 2.25-mg/kg S application. The results revealed that farmyard manure biochar and Planomicrobium sp. strain MSSA-10 in combination with 2.25-mg/kg S proved effective for the management of charcoal rot disease through regulating the antioxidant enzymes’ activities and strengthening the immune system of sunflower plants. The studied health markers (total chlorophyll content and carotenoids) and stress markers (total protein content, catalase and peroxidase) were significantly altered by the applied treatments under 0 and 2.25-mg/kg S applications. The findings of the experiment indicated that both farmyard manure biochar and Planomicrobium sp. strain MSSA-10, combined with 2.25-mg/kg S, could be used to enhance the crop yield and manage charcoal rot disease in sunflowers. Farmyard manure biochar and Planomicrobium sp. strain MSSA-10 are an easy-to-apply, cost-effective, ecofriendly and sustainable option for the management of charcoal rot disease in sunflowers.

Keywords: antioxidants; Macrophomina phaseolina; sulfur; PGPR; biochar
1. Introduction

Sunflower (Helianthus annuus L.) belongs to the family Asteraceae and genus Helianthus, which consists of sixty-five species [1]. It is third-most important oilseed crop and mostly cultivated in temperate and subtropical zones of the world [2]. Sunflowers are successfully cultivated in irrigated and rainfed regions of Pakistan [3]. Sunflowers are cultivated during two distinct seasons, i.e., the summer and spring; thus, they can be rotated with other crops, including potatoes, rice or cotton [4]. During 2019 to 2020, sunflowers were cultivated on 219,000 hectares, which produced 105,000 tons of seeds and 40,000 tons of oil [5].

Sunflower production is negatively affected by several biotic and abiotic factors, including disease infestation. Sunflowers are infested by 90 different diseases caused by several microorganisms [6]. Charcoal rot caused by Macrophomina phaseolina (Tassi) Goid is a significant threat to sunflower production around the world [7–9]. Macrophomina phaseolina can be seed-, soil- or stubble-borne and has a wide range of wild and cultivated host species [10]. A total of 67 host species for M. phaseolina have been reported from Pakistan [11]. Macrophomina phaseolina was firstly reported in Sri Lanka in 1927 [11], while it was reported in Pakistan in 1982 in Faisalabad District [12]. Afterwards, the disease spread to different areas of the Sindh, Punjab and Khyber Pakhtunkhwa Provinces of Pakistan. Different morphological and physiological attributes of sunflowers, including plant height, stem and head diameter, are negatively affected by charcoal rot disease. The disturbed physiological and morphological attributes reduce the yield, and yield reduction may reach up to 90% under high infestation [11,13].

The management of charcoal rot is a challenging task, as disease infestation is influenced by numerous factors [14]. Only a few management options are available for the management of charcoal rot once the infestation starts in the field [15,16]. Charcoal rot disease in Pakistan is managed by the use of resistant cultivars and synthetic fungicides [17,18]. The fungicides are either seed-treated or soil-applied for the management of charcoal rot. However, seed treatment is not effective for the long term, and soil application incurs heavy economic costs [19]. Nevertheless, the excessive use of synthetic fungicides led to the evolution of resistance in pathogenic strains [9,20,21]. Furthermore, the application of synthetic fungicides causes environmental pollution and affects soil microbial communities. Therefore, ecofriendly alternative management options are inevitable for the management of charcoal rot disease [22,23].

Recently, plant growth-promoting rhizobacteria (PGPR) have been recommended for the management of soil-borne diseases. These PGPRs include Azotobacter, Azospirillum and Klebsiella, all of which are used to improve the crop productivity [24,25]. The PGPRs compete with the pathogens in dual ways, i.e., either compete for nutrients or produce antibiotics, hydrogen cyanide and siderophores, all of which suppress pathogens [19,24,26]. The PGPRs are ecofriendly and can be effectively used for suppressing plant pathogens. Khare and Arora [27] recently reported that the biosurfactant-based formulation of Pseudomonas guariconensis effectively controlled the charcoal rot of sunflowers. Nonetheless, PGPRs have been proved effective in controlling charcoal rot in soybeans [28], strawberies [29], common beans [30], sorghum [31–33], chickpeas [34] and sunflowers [25,34,35]. However, these PGPRs have not been combined with macronutrients and biochar for inferring their combined effects on charcoal rot suppression.

Mineral nutrition has recently been manipulated for the management of plant diseases [21,36]. Farmyard manure and the application of sulfur (S) have been proven effective in controlling charcoal rot [37]. Sulphur is the fourth-most important nutrient in crop production after nitrogen (N), phosphorus (P) and potassium (K). Sulfur-containing amino acids (cystin, methionine and methionine) are essential for chlorophyll synthesis [38]. Moreover, S had a significant influence on the chemical composition of seeds [39]. The application of 25-kg/ha S increased the dry biomass production, seed production (up to 55 kg/ha), oil percentage (45%), 1000-grain weight, shoot length and head diameter of sunflowers. Fungicidal impacts of S were reported at the end of the 19th century against...
a variety of pathogens [40]. However, the management of charcoal rot of sunflowers has been done through silicon application [21,35], and S is often ignored in this regard.

Biochar is a carbon-rich material and obtained through the pyrolysis of different compounds, including farmyard manure, plant parts and other substances [41]. The application of biochar increases crop productivity and soil fertility [42–45]. Biochar produced from different substances have proven effective in suppressing the proliferation of casual organisms of different diseases [46,47]. However, the impacts of farmyard manure biochar remain unexplored in the suppression of charcoal rot in sunflowers.

Although the impacts of different PGPRs, mineral nutrition and biochar have been explored on disease suppression individually, their interactive impacts remain unexplained. Therefore, the current study aimed to: (i) evaluate charcoal rot infestation under natural conditions; (ii) investigate the activation of antioxidant enzymes and detoxifying reactive oxygen species to strengthen the plant defense mechanism by inducing a systemic acquired resistance against *M. phaseolina* and (iii) find the impacts of S alone or in combination with farmyard manure biochar, PGPRs and macronutrients to manage charcoal rot disease in sunflowers. It was hypothesized that the combined application of S, PGPRs and farmyard manure biochar will suppress charcoal rot infestation and improve sunflower productivity. The results of the study will help to manage the charcoal rot of sunflowers in an ecofriendly and sustainable manner.

2. Materials and Methods

2.1. Collection of Experimental Material

A pot study was conducted in the research area of the College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah, Punjab, Pakistan during 2019-2020. Seeds of the sunflower variety (“PAC-47”) were obtained from the National Agriculture Research Center (NARC), Islamabad, Pakistan. The pure culture of *Macrophomina phaseolina* was obtained from the Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of Punjab, Lahore, Pakistan and multiplied in the laboratory. Plant growth-promoting rhizobacteria (PGPR) strains, i.e., *Bacillus* sp. strain MR-1/2 (accession number MG548383), *Achromobacter* sp. strain FB-14 (accession number MG547707) and *Planomicrobium* sp. strain MSSA-10 (accession number KU672730), were taken from the Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan [48,49]. The NPK fertilizer brand name “Soloplant” (20:20:20) (Jaffer Group of Companies, Pakistan) and S brand name “Sulphur” (80% S) were procured from a local market. Plastic pots with a 25-L capacity were procured from Model Nursery Layyah. Farmyard manure for biochar production was collected from Hussain Dairy Farm Layyah, Pakistan. Farmyard manure was first oven-dried at 70 °C until a constant weight, followed by pyrolyzation using a Muffle furnace (BenchTop Muffle Furnace—1230F28, Thomas Scientific, Inc., NJ, The United States) following Qayyum et al. [50]. Gasses and vapors from the working area were removed by a glass rod outlet, where silicon grease was used to seal the junction of the glass rod and Pyrex flask. The material was pyrolyzed at 300 °C for 20 min in the reaction chamber, followed by cooling of the furnace and biochar removal.

2.2. Experimental Layout

The experiment was laid out according to a completely randomize design with factorial arrangement. The sulfur application was taken as the main factor, whereas the remaining treatments, including *M. phaseolina*, farmyard manure biochar and PGPRs, were treated as a subfactor. All treatments had three replications. Treatments used to assess the effects of charcoal rot disease management are given in Table 1.
Table 1. The experimental treatments used in the study to manage the charcoal rot disease of sunflowers.

| Sulphur Dose (mg/kg) | MP | Biochar + MP | NPK + MP | PGPR1 + MP | PGPR2 + MP | PGPR3 + MP |
|----------------------|----|-------------|----------|------------|------------|------------|
| 0.00 T                | T1 | T2          | T3       | T4         | T5         | T6         |
| 2.25 T               | T7 | T8          | T9       | T10        | T11        | T12        |

Here: MP = *Macrophomina phaseolina*, PGPR1 = *Bacillus* sp. strain MR-1/2, PGPR2 = *Achromobacter* sp. strain FB-14, PGPR3 = *Planomicrobium* sp. strain MSSA-10 and Biochar = Farmyard manure biochar.

2.3. Crop Husbandry

The pots were filled with 20-kg soil, and five seeds were sown in each pot. The NPK at 20:20:20 mg/kg was applied at the time of sowing. The sulfur solution was prepared by adding 20 g of “Sulphur” in 980 mL of water and applied to the pots. Farmyard manure biochar (400 g) was applied to each pot before sowing. Each pot was treated with 30 mL of the relevant PGPR solution according to the treatments. The recommended agronomic practices by the Agriculture Extension Department, Government of Punjab, Pakistan for sunflower crops were opted for throughout the experiment.

2.4. Data Collection

2.4.1. Disease and Growth Assessments

The plants were examined to observe the disease severity and plant mortality in each pot of each replication. The plants in each pot were observed regularly for the typical symptoms caused by *M. phaseolina* (Table 2).

Table 2. Disease rating scale according to the disease symptoms appearing on sunflower plants.

| Rating | Disease Symptoms                              | Resistance Level       |
|--------|----------------------------------------------|------------------------|
| 1      | No disease sign observed                      | Extremely resistant     |
| 3      | Slight cuts on cotyledon tissue               | Resistant              |
| 5      | Cuts developed on cotyledon                  | Tolerant               |
| 7      | Widespread cuts on branches and stem          | Susceptible            |
| 9      | Maximum growing points were infested         | Highly susceptible      |

Plant mortality was recorded 40 days after sowing. Data regarding the head formation, seed germination per pot, head injury, head diameter (cm), number of leaves per plant, shoot length (cm), fresh shoot weight (g), stem diameter (cm), shoot dry weight (g), root dry weight (g) and 100-seeds weight were recorded at physiological maturity of the crop. Measurements for these traits were made from all plants in each pot of every replication, and then, the average was computed.

2.4.2. Assessment of Physio-Chemical Attributes

Leaf samples were collected 35 days after sowing and kept in Eppendorf tubes containing liquid nitrogen for the physiochemical analysis. The plant samples (stored in freezing conditions) were mixed in a 1:3 (*w/v*) concentration with 0.1-M HCl buffer (pH-7.0) and mixed thoroughly. The homogenized mixture was filtered and centrifuged for 10 min at 0 °C. Afterwards, the supernatant was taken out, and the residue materials were saved for the protein extraction. Protein was precipitated by the addition of ammonium sulphate [(NH₄)₂SO₄] for 30 min at 0 °C. The total protein content was measured by following the protocol of Lowry et al. [51]. The total chlorophyll content was assessed according to Wellburn [52]. For measuring the catalase activity, 50-mM phosphate buffer (pH 7.8) and 100-mM H₂O₂ solution was preheated at 25 °C in a water bath. The 2.5 mL of 0.2-M phosphate buffer and 0.2-M enzyme solution was added to a 10-mL tube heated at 25 °C in a water bath for 3 min, followed by the addition of 0.3 mL of 100-mM H₂O₂ solution. Immediately after mixing, the absorbance at 240 nm was measured at intervals of 1 min. The catalase activity was measured by following Calmak and Horst [53]. Similarly, the
polyphenol oxidase activity was assessed according to Kumar and Khan [54]. The carotene pigments were extracted with petroleum ether and alcohol (45%), to which sodium hydroxide (NaOH) was added. The contents of the carotene pigments were read at a wavelength of 448 µm for the measurement of the carotenoids [55].

2.5. Statistical Analysis

Collected data regarding the morphological and biochemical attributes were tested for normality by the Shapiro–Wilk normality test [56]. The data were normally distributed; therefore, an analysis was performed on the original data. A two-way analysis of variance (ANOVA) was used to measure the significance in the data. A least significant difference test at \( p \leq 0.05 \) was used to separate the means where the ANOVA indicated significant differences among the applied treatments [57]. The mean interactive effects of the applied treatments were presented and interpreted in the manuscript.

3. Results

3.1. Disease Incidence and Plant Mortality

The inoculation of \( M. \) phaseolina without S application resulted in the highest disease infestation and plant mortality, whereas NPK without S application recorded the similar disease incidence and plant mortality. The lowest disease incidence and plant mortality were recorded for 2.25-mg/kg S application combined with farmyard manure biochar and \( \text{Planomicrobium} \) sp. strain MSSA-10 (Figure 1).

![Figure 1](image-url)

**Figure 1.** The impact of sulfur application combined with NPK, plant growth-promoting rhizobacteria and farmyard manure biochar on the charcoal rot incidence (%) and mortality (%) of sunflower plants. In the x-axis, MP = \( \text{Macrophomina phaseolina} \), biochar = farmyard manure biochar, PGPR1 = \( \text{Bacillus} \) sp. strain MR-1/2, PGPR2 = \( \text{Achromobacter} \) sp. strain FB-14 and PGPR3 = \( \text{Planomicrobium} \) sp. strain MSSA-10.

3.2. Impact of Different Treatments on Growth Attributes of Sunflower

The sulfur application had a nonsignificant \( (p \leq 0.05) \) impact on the seed germination of sunflower. \( \text{Macrophomina phaseolina} \) reduced the seed germination, while the application of farmyard manure biochar and PGPRs improved the seed germination under no and 2.25-mg/kg S application (Table 3).
Table 3. The impact of sulfur application and different treatments used in the study on the growth attributes of sunflowers.

| Parameters                              | Sulfur mg/kg | MP       | Biochar + MP | NPK + MP | PGPR1 + MP | PGPR2 + MP | PGPR3 + MP |
|-----------------------------------------|--------------|----------|-------------|----------|------------|------------|------------|
| Germination (seeds/pot)                 | 0            | 3.66 a–c | 4.66 a      | 2.66 c   | 4.66 a     | 3.66 a–c   | 3.00 bc    |
|                                         | 2.25         | 3.66 a–c | 4.00 ab     | 3.33 bc  | 3.66 a–c   | 4.66 a     | 2.66 c     |
| Head formation (number of plants)       | 0            | 3.00 b   | 3.33 ab     | 1.33 c   | 3.00 b     | 2.33 bc    | 2.33 bc    |
|                                         | 2.25         | 3.33 ab  | 3.00 b      | 2.66 b   | 2.66 b     | 4.33 a     | 1.33 c     |
| Head injury (number of heads injured)   | 0            | 4.33 a   | 2.33 bc     | 3.33 ab  | 2.33 bc    | 2.00 c     | 1.66 c     |
|                                         | 2.25         | 3.33 ab  | 1.66 c      | 2.33 bc  | 2.33 bc    | 1.33 c     | 1.33 c     |
| Shoot length (cm)                       | 0            | 67.77 g  | 74.00 d–f   | 81.22 b  | 70.22 fg   | 82.77 ab   | 73.61 d–f  |
|                                         | 2.25         | 76.88 cd | 86.44 a     | 81.11 b  | 75.88 de   | 80.44 bc   | 76.88 cd   |
| Total number of leaves/plants           | 0            | 13.44 c–e| 12.66 e     | 15.22 a–c| 12.77 e    | 13.44 c–e  | 13.66 c–e  |
|                                         | 2.25         | 13.33 c–e| 17.00 a     | 16.55 ab | 14.88 bc   | 13.00 de   | 16.66 ab   |
| Stem diameter (cm)                      | 0            | 0.20 c–e | 0.19 e      | 0.23 a–c | 0.19 e     | 0.20 c–e   | 0.21 c–e   |
|                                         | 2.25         | 0.20 c–e | 0.26 a      | 0.23 a–c | 0.22 b–d   | 0.20 de    | 0.25 ab    |
| Shoot fresh weight (g)                  | 0            | 87.22 d  | 90.56 cd    | 97.89 bc | 90.78 cd   | 106.44 a   | 94.11 cd   |
|                                         | 2.25         | 105.11 ab| 109.33 a    | 109.22 a | 110.44 a   | 112.44 a   | 104.89 ab  |
| Shoot dry weight (g)                    | 0            | 16.33 ab | 16.00 a–c   | 15.0 b–e | 15.33 a–d  | 16.33 ab   | 14.33 c–f  |
|                                         | 2.25         | 17.00 a  | 14.00 d–f   | 13.33 ef | 15.66 a–d  | 15.00 b–e  | 12.66 f    |
| Root dry weight (g)                     | 0            | 3.05 b–d | 2.26 ef     | 2.73 de  | 2.83 c–e   | 2.41 d–f   | 3.05 b–d   |
|                                         | 2.25         | 2.20 ef  | 3.04 b–d    | 3.51 a–c | 3.76 ab    | 4.17 a     |           |
| 100-seed weight (g)                     | 0            | 7.62 de  | 7.66 de     | 7.62 de  | 7.75 c–e   | 7.47 e     | 7.80 b–d   |
|                                         | 2.25         | 8.09 ab  | 8.14 a      | 7.99 a–c | 8.08 ab    | 7.80 b–d   | 8.20 a     |

Any of the two treatments that do not share the same letter within a column or a row are statistically different from each other at $p \leq 0.05$. MP = Macrophomina phaseolina, biochar = farmyard manure biochar, PGPR1 = Bacillus sp. strain MR-1/2, PGPR2 = Achromobacter sp. strain FB-14 and PGPR3 = Planomicrobium sp. strain MSSA-10.

The head formation was significantly ($p \leq 0.05$) influenced by the applied treatments. The sulfur application combined with PGPR2 significantly ($p \leq 0.01$) improved the head formation. The highest head formation was observed for PGPR2 combined with the 2.25-mg/kg S application. The lowest head formation was recorded for *M. phaseolina* under no S application and *M. phaseolina* + PGPR3 with 2.25-mg/kg S application (Table 3).

The sulfur application (2.25 mg/kg) significantly ($p \leq 0.01$) reduced head injuries. The highest head injury was recorded for *M. phaseolina* inoculation without the S application, whereas PGPR2 and PGPR3 with or without the S application and farmyard manure biochar with a 2.25-mg/kg S application resulted in the lowest head injury (Table 3).

Sulfur application significantly ($p \leq 0.01$) improved the shoot lengths of the sunflowers. The applied treatments under no S application recorded shorter shoots produced compared to 2.25-mg/kg S application. The inoculation of *M. phaseolina* under no S application recorded the shortest shoots, whereas farmyard manure biochar combined with 2.25-mg/kg S application recorded the longest shoot length (Table 3).

The number of leaves were significantly ($p \leq 0.01$) altered by S application and the applied treatment interactions. All the treatments produced a lower number of leaves per plant under no S application compared to the 2.25-mg/kg S application. The lowest number of leaves per plant was noted for PGPR1 under no S application, while farmyard manure biochar combined with 2.25-mg/kg S application produced the highest number of leaves per plant (Table 3).

The stem diameter was significantly ($p \leq 0.01$) influenced by the interactive effect of the applied treatments and S application. A higher stem diameter was recorded for the treatments combined with 2.25-mg/kg S application compared with no S application. Farmyard manure biochar and PGPR1 combined with no S application resulted in the lowest stem diameter, while farmyard manure biochar combined with 2.25-mg/kg S application resulted in the highest stem diameter (Table 3).
The application of 2.25-mg/kg S combined with the applied treatments improved the shoot fresh weight compared to no S application. Farmyard manure biochar, NPK, PGPR1 and PGPR2 under 2.25-mg/kg S application and PGPR2 under no S application resulted in the highest shoot fresh weight. The inoculation of *M. phaseolina* under no S application produced the lowest shoot fresh weight (Table 3).

The shoot dry weight was improved by the application of 2.25-mg/kg S, while a reduction in shoot dry weight was also noted with the 2.25-mg/kg S application. The highest and the lowest shoot dry weights were noted for *M. phaseolina* inoculation and PGPR2 under the 2.25-mg/kg S application (Table 3).

The root dry weight was improved by the application of 2.25-mg/kg S combined with different treatments used in the study. The highest root dry weight was recorded for PGPR3 combined with the 2.25-mg/kg S application, whereas NPK applied under no S supplementation recorded the lowest root dry weight (Table 3).

The sulfur application (2.25 mg/kg) in combination with the applied treatments improved the 100-seed weight compared to the no S application. All of the applied treatments produced higher 100-seed weights under the 2.25-mg/kg S application compared with the no S supplementation. Farmyard manure biochar and PGPR3 combined with 2.25-mg/kg S application recorded the highest 100-seed weight, whereas PGPR2 with no S application resulted in the lowest 100-seed weight (Table 3).

### 3.3. Impact of Various Treatments on Physiochemical Attributes of Sunflowers

The sulfur application significantly (*p* ≤ 0.05) improved the polyphenol oxidase (PPO) and catalase activity. Higher catalase and PPO activities were recorded for NPK combined with the 2.25-mg/kg S application. The lowest catalase and PPO activities were recorded for *M. phaseolina* inoculation under no S application (Table 4).

**Table 4.** The impacts of the sulfur application and other treatments used in the study on the physiochemical attributes of sunflowers.

| Parameters | Sulfur mg/kg | MP | Biochar + MP | NPK + MP | PGPR1 + MP | PGPR2 + MP | PGPR3 + MP |
|------------|--------------|----|--------------|----------|------------|------------|------------|
| polyphenol oxidase activity (min⁻¹ mg⁻¹ of protein) | 0 | 0.054 f | 0.051 f | 0.15 bc | 0.091 d | 0.084 de | 0.17 b |
| | 2.25 | 0.089 d | 0.083 de | 0.23 a | 0.14 c | 0.063 ef | 0.095 d |
| Catalase activity (min⁻¹ mg⁻¹ of protein) | 0 | 11.98 g | 23.34 de | 27.98 bc | 18.31 f | 19.39 ef | 29.99 b |
| | 2.25 | 16.18 f | 25.81 cd | 36.05 a | 22.70 de | 29.61 bc | 18.60 f |
| Carotenoids (mg g⁻¹ FW) | 0 | 2.26 b | 1.69 d-f | 2.11 bc | 1.88 c-e | 2.63 a | 1.74 de |
| | 2.25 | 1.99 b-d | 1.38 f | 2.18 bc | 1.89 c-e | 1.64 ef | 2.12 bc |
| Protein (mg g⁻¹ FW) | 0 | 0.13 a | 0.07 cd | 0.10 b | 0.08 c | 0.07 cd | 0.08 cd |
| | 2.25 | 0.07 cd | 0.07 cd | 0.06 de | 0.07 de | 0.05 e | 0.08 c |
| Total chlorophyll (mg g⁻¹ FW) | 0 | 0.410 bc | 0.292 d | 0.460 ab | 0.372 c | 0.446 ab | 0.460 ab |
| | 2.25 | 0.419 a-c | 0.423 a-c | 0.464 ab | 0.483 ab | 0.487 a | 0.475 ab |

Any of the two treatments that do not share the same letter within a column or a row are statistically different from each other at *p* ≤ 0.05. MP = *Macrophomina phaseolina*, biochar = farmyard manure biochar, PGPR1 = *Bacillus* sp. strain MR-1/2, PGPR2 = *Achromobacter* sp. strain FB-14 and PGPR3 = *Planomicrobium* sp. strain MSSA-10.

Sulfur application combined with the applied treatments significantly (*p* ≤ 0.05) influenced the carotenoids. The carotenoids were higher under no S application compared to the 2.25-mg/kg S application. The highest carotenoid contents were noted for PGPR2 under no S application, whereas farmyard manure biochar under 2.25-mg/kg S resulted in the lowest carotenoid contents (Table 4).

The protein contents were higher (*p* ≤ 0.01) under no S application. The highest protein contents were recorded for the *M. phaseolina* inoculation under no S application, whereas PGPR2 applied under 2.25-mg/kg S recorded the lowest protein contents (Table 3).

Sulfur had a significant impact (*p* ≤ 0.05) on the total chlorophyll content of the sunflowers. Higher total chlorophyll contents were observed for all treatments under
the 2.25-mg/kg S application compared to no S application. The PGPR3 combined with 2.25-mg/kg S application resulted in the highest total chlorophyll contents, while farmyard manure biochar under no S application resulted in the lowest chlorophyll contents (Table 4).

4. Discussion

Charcoal rot is of paramount importance around the globe and negatively affects the stem height, weight and girth and root weight of sunflower plants [8,11,21,35]. High temperature and low moisture make the disease more severe and result in heavy economic losses [58,59]. Charcoal rot negatively affects the growth and yield of soybeans [28], strawberries [29], common beans [30], sorghum [31–33], chickpeas [34] and sunflowers [25,34,35] globally. Similarly, the severity of the disease differs among various hosts [60].

The current study exhibited that the application of S enhanced the growth, yield and physiochemical attributes of sunflowers. As hypothesized, the applied treatments differed significantly for their potential in disease suppression. In some cases, S application seemed to negatively affect the growth of sunflowers; however, overall, it reduced the disease incidence and improved growth of sunflower plants. A reduced yield of sunflowers with an application of S in some cases might be due to an iron and manganese deficiency [61,62] or S toxicity. Sulfur is vital for the maintenance of membranes [38].

Different PGPRs, biochar and NPK positively affected the morphological and biochemical attributes of sunflowers. The PGPRs probably competed with the pathogens in dual ways, i.e., either by competing for nutrients or producing antibiotics, hydrogen cyanide and siderophores, all of which suppress pathogens [19,24,26]. However, these were not measured in the current study; therefore, they should be considered in future studies. The impacts of PGPRs, biochar and NPK on the morphological and biochemical attributes were more pronounced when these were combined with 2.25-mg/kg S. The differences in the potential of different PGPRs in disease suppression were revealed through this study. Planomicrobium sp. strain MSSA-10 PGPR resulted in the highest suppression of charcoal rot. This suppression can be linked to improved mineral nutrition, and a recent study revealed that this PGPR improved the growth and nutrient availability for pea plants [49]. Therefore, different PGPRs must be used in disease suppression in future studies. Nonetheless, a mixture of different PGPRs have given promising results in disease suppression [25,63]. Therefore, the impact of the mixtures of different PGPRs on charcoal rot suppression must be explored in future studies.

The sulfur application influenced the yield parameter, i.e., seed weight, whereas it had no impact on the head diameter and plant height. The diminished anther and pollen development due to reduced indole acetic acid and proteins might be responsible for the decrease in these traits [61,62]. The farmyard manure biochar and NPK treatments enhanced the different growth attributes and suppressed the incidence of charcoal rot, which can be attributed to the positive impact of soil nutrients and moisture presence induced by biochar. Biochar has a high porosity [64], which expands the surface area, resulting in increased nutrient binding [64,65]. Various studies have revealed that biochar restricts N leaching and enhances the N-use efficacy [66,67]. Biochar produced from different substances have been proven effective in suppressing the proliferation of casual organisms of different diseases [46,47]. The increased growth and disease suppression by the application of farmyard manure biochar can be explained with higher nutrient and moisture availability. The increased availability of nutrients and water made the plants competitive and enabled them to cope with disease infestation.

5. Conclusions

In crux, the current study revealed that S application combined with farmyard manure biochar and Planomicrobium sp. strain MSSA-10 PGPR could be helpful in the management of charcoal rot disease and enhance various growth, yield and physiochemical attributes of sunflowers. However, further research is required under in vivo environments for the confirmation of the actual mechanisms involved in disease suppression.
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