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RESEARCH ARTICLE

Utilization of Biochar and Biocontrol Agents for The Effective Management of Seed-Borne Pathogens in Mung Bean

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ABSTRACT

Mung bean crops are frequently threatened by seed-borne pathogens, which can significantly affect plant growth and yield. This study evaluates the effectiveness of biochar and biocontrol agents in controlling these pathogens. Seeds from six different mung bean varieties, AZRI Mung 2021, Mung NM-1, JUMBU Mung, Mung NM-16, Abbas Mung, and Mung 2021, were obtained from the Pulses Program at the Crop Sciences Institute, National Agricultural Research Centre, Islamabad. Seed infection percentages were assessed across these varieties to identify the most susceptible one. The most vulnerable variety was selected and sown in 40 pots using eight different treatments, each replicated five times. The treatments included biochar derived from cannabis leaves and biocontrol agents such as *Trichoderma harzianum* and *Bacillus subtilis*. Both laboratory and field evaluations were conducted to assess the effectiveness of these treatments on parameters including plant growth, disease incidence, and severity. The results demonstrated that biochar and biocontrol agents significantly reduced disease incidence and enhanced plant health. This study highlights the potential of these eco-friendly and sustainable approaches for managing seed-borne pathogens in mung bean cultivation, contributing to improved crop performance and yield.

Keywords: Mung bean, Seed-borne pathogens, Biochar, Biocontrol agents, *Trichoderma harzianum*, *Bacillus subtilis*.

INTRODUCTION

Pulses are a crucial source of plant-based protein in Pakistan, occupying about 5% of the total cropped area, which amounts to approximately 1.5 million hectares. They play a vital role in a balanced diet, offering 20-24% protein content, 2 to 3 times higher than that of cereals. With the growing population, there is an urgent need to increase pulse production to meet dietary protein demands (Ashfaq *et al.*, 2014; Iqbal and Mukhtar, 2014; Mukhtar *et al.*, 2017).

The term “bean” is often used interchangeably with

“pulse” and refers to edible legumes belonging to the Fabaceae (Leguminosae) family. Beans are valuable not only for human consumption and animal feed but also for improving soil fertility. With a protein content ranging from 6-22%, beans provide an affordable alternative to animal protein, particularly for low-income populations in developing countries (Iranga *et al.*, 1985).

Mung bean (*Vigna radiata*) is a significant pulse crop in Asia, especially in India, Southeast Asia, and East Asia, where it complements cereal-based diets by providing an

additional source of protein (Lambrides *et al.*, 2007). Mung bean is an inexpensive, nutrient-rich crop (Aguilera *et al.*, 2013) that plays a vital role in regional agriculture, particularly through its ability to fix atmospheric nitrogen symbiotically (Siddiqui *et al.*, 2001).

Globally, pulses account for 12-15% of arable land, contribute 27% to total crop output, and supply 33% of dietary protein. Their high protein content makes them a critical nutritional resource for millions of people (Mishra *et al.*, 2021).

Although the area under cereal and pulse cultivation has generally declined over the past two decades, mung bean acreage has doubled, expanding at an annual growth rate of 2.5%. In India alone, pulses are cultivated on about 24.38 million hectares, yielding 14.52 million tons. Mung bean ranks as the third most important pulse crop in India after pigeon pea and gram (Sathyamoorthi *et al.*, 2008).

Biocontrol of plant pathogens involves the use of beneficial microorganisms such as bacteria, fungi, or their metabolites to suppress disease-causing organisms (Iqbal and Mukhtar, 2022; Saeed *et al.*, 2023; Aziz *et al.*, 2024; Palomino-Malpartida *et al.*, 2025; Wassan *et al.*, 2025). These biocontrol agents work through various mechanisms, including competition for nutrients and space, production of antimicrobial compounds, parasitism, and induction of plant systemic resistance (Mukhtar *et al.*, 2013, 2021; AL-Ajeely *et al.*, 2025; Shahbaz *et al.*, 2025). Biocontrol offers an eco-friendly and sustainable alternative to chemical pesticides for managing plant diseases (Ahmed *et al.*, 2025; Jamel *et al.*, 2025; Zubairi *et al.*, 2025).

Seed treatment with fungicides, plant extracts, and bioagents can effectively suppress seed-borne fungal infections (Erdey *et al.*, 1997; Iqbal and Mukhtar, 2020). The current study aims to evaluate the efficacy of such seed treatment techniques in reducing mung bean seed-borne pathogens and improving seed quality.

Combining one biocontrol agent (BCA) with two application methods has proven more effective than using two different BCAs with separate strategies. A higher penetration rate of a single BCA likely results in greater disease suppression. However, when a mycoparasitic BCA and an antibiotic-producing BCA were used together, their combined effect did not significantly alter disease dynamics (Elad, 2000).

Biochar, produced through the pyrolysis of plant material, crop residues, and animal waste in an oxygen-limited environment, is a carbon-rich, fine-grained substance

(Zheng *et al.*, 2010). As a stable organic material derived from agricultural waste, biochar can reduce soil-borne and foliar fungal infections by influencing the activity of beneficial microorganisms, altering root exudates, modifying soil structure, and regulating nutrient availability (Poveda *et al.*, 2021).

Biochar also reduces nutrient leaching, minimizing groundwater contamination (Yu *et al.*, 2009). It strengthens root defense mechanisms, enhances stress hormone signaling, and reduces oxidative stress by modulating reactive oxygen species (ROS). These effects collectively trigger systemic resistance against pathogens. Furthermore, biochar improves soil microbial communities, which can suppress disease by interfering with pathogen mobility and colonization (Frenkel *et al.*, 2017).

Although other organic amendments, such as compost, poultry manure, and farmyard manure (FYM), can enhance soil organic matter, biochar stands out for its stability and high carbon content. This has attracted widespread scientific interest (Lehmann *et al.*, 2011). While biochar and conventional organic matter share similarities (Wolf *et al.*, 2008), biochar's higher carbon content makes it particularly suitable for use in legume cultivation (Rahim *et al.*, 2019). It improves the physical, biochemical, and biological properties of degraded soils, thereby supporting healthy plant growth.

Given these advantages, the present study was undertaken to assess the efficacy of biochar and biocontrol agents in managing seed-borne diseases in mung bean, and to compare the effects of different treatments on mung bean growth and disease-related parameters.

MATERIALS AND METHODS

Seed Source and Experimental Setup: Seeds of six mung bean varieties, AZRI Mung 2021, Mung NM-1, JUMBO Mung, Mung NM-16, Abbas Mung, and Mung 2021, were obtained from the Pulses Program at the Crop Sciences Institute, National Agricultural Research Centre (NARC), Islamabad. These seeds were subjected to comprehensive testing for seed-borne pathogens in both laboratory and greenhouse settings.

Identification and Isolation of Seed-Borne Pathogens in Mung Bean Varieties: Sterilization was carried out by autoclaving distilled water and filter paper for 15 minutes at 15 psi. The autoclaved water was then allowed to cool to room temperature. Three sterilized Petri dishes were prepared: one containing 2% Clorox and two containing

distilled water. Seeds from each mung bean variety were divided into three groups. For each group, seeds were soaked in 2% Clorox for one minute and then rinsed in two successive Petri dishes with distilled water for 30 seconds each. Sterile forceps were used throughout the process. The disinfected seeds were dried on sterilized filter paper and then transferred onto Potato Dextrose Agar (PDA) medium in Petri dishes. The plates were sealed with parafilm, labeled, and incubated at 25°C. Plates were monitored for signs of fungal growth or discoloration, and the presence of seed-borne pathogens was recorded following the methodology reported by Singh *et al.* (2014).

Evaluation of Seed Infection Percentage: The seed infection percentage is a critical parameter for assessing the health and viability of mung bean seeds. This study evaluated seed-borne pathogen infection on PDA medium across six mung bean varieties to determine their susceptibility and support the development of effective disease management strategies (Singh *et al.*, 2014). The following formula was used to calculate seed infection percentage:

$$\text{Seed infection \%} = \frac{\text{Number of infected seeds}}{\text{Total number of treated seeds}} \times 100$$

Purification of Fungal Pathogens from Culture Media:

Fungal pathogens were purified following the protocol of Solanki *et al.* (2018), with minor modifications. PDA medium was prepared by dissolving PDA powder in distilled water and sterilized at 121°C for 15-20 minutes at 15 psi. The sterilized medium was poured into Petri dishes and allowed to cool and solidify. Distinct fungal colonies grown on the original PDA plates were transferred using a sterile inoculation loop to fresh PDA plates. These were incubated at 25-28°C for 5-7 days until substantial fungal growth was observed.

Microscopy and Slide Preparation: Microscopic identification of fungal sporulation followed the procedure of Deshmukh *et al.* (2016). After 5-7 days of incubation, a drop of lactophenol blue was placed on a clean glass slide under a laminar flow hood. Spores from the fungal fruiting bodies of NM-16 and JUMBO Mung samples were gently scraped using a sterile needle and transferred onto the slide. A sterile coverslip was placed over the spores, avoiding air bubbles, and gently pressed to ensure even distribution. The slide was air-dried completely and examined under a microscope. The identified pathogens were *Fusarium* from NM-16 and *Trichoderma harzianum* from JUMBO Mung.

Pot Preparation and Seed Sowing Techniques: The experiment was conducted at the University Research Farm (URF), Koont, during the mung bean growing season. The aim was to assess the efficacy of biochar and biocontrol agents in managing leaf spot disease in mung bean. Forty pots were prepared in a greenhouse using the susceptible variety NM-16. Each pot was filled with a soil mixture composed of organic matter, peat moss, soil, and sand in a 1:1:2:1 ratio. Five seeds were sown per pot. Upon seedling emergence, eight treatments were applied: Difenconazole (fungicide), Biochar Soil Drench (BSD), Biochar Top Dressing (BTD), Biochar Potting Mix (BPM), and biocontrol agents *Bacillus subtilis* (BaS) and *Trichoderma harzianum* (TrH), along with positive and negative controls. Each treatment was replicated five times. Forty-eight hours after treatment, a pathogen suspension was inoculated onto the leaves. Biochar was prepared from cannabis leaves and applied in various formulations to evaluate its effectiveness. Plants were maintained under optimal conditions for pathogen development.

Inoculation and Treatment Application: Biochar derived from cannabis leaves and biocontrol agents (*T. harzianum* and *B. subtilis*) were applied as treatments. Forty-eight hours' post-treatment, a suspension containing seed-borne pathogens was inoculated onto the leaves. Plants were kept under controlled conditions to encourage pathogen development. At plant maturity, data were collected on parameters including number of leaves, plant height, shoot and root weight, disease incidence, and disease severity.

STATISTICAL ANALYSIS

Data were analyzed using Statistix 8.1 software. A Completely Randomized Design (CRD) was used for the experiment. Treatment means were compared using a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Identification and Isolation of Seed-Borne Pathogens

Seed-borne pathogens have a substantial impact on crop health by reducing germination rates, inhibiting plant growth, and ultimately lowering yield. In this study, six mung bean varieties were analyzed using PDA medium to isolate and identify seed-borne pathogens. After a three-day incubation period, fungal cultures were purified and examined at the Fungal Plant Pathology Laboratory. The isolation process revealed that *Fusarium* species were predominantly associated with the NM-16 variety. This finding underscores the susceptibility of NM-16 to

Fusarium infection, indicating the need for targeted disease management strategies for this variety.

Evaluation of Seed Infection Percentage

The evaluation of seed infection percentages across the different mung bean varieties is summarized in Table 1. This analysis provides a comparative overview of seed-borne pathogen infection rates among the varieties. The infection percentage and the corresponding number of fungal infections for each variety were calculated using the seed infection percentage formula described by Singh *et al.* (2014).

The evaluation revealed significant differences in susceptibility to seed-borne fungal infections among

the six mung bean varieties. Mung NM-16 and Abbas Mung were the most susceptible, exhibiting high infection rates of 53% and 46%, respectively, both accompanied by severe disease severity. Azri Mung 21 and Mung NM-21 showed moderate susceptibility, with infection rates of 33% and 20% and corresponding moderate disease severity. In contrast, Jumbo Mung and Mung NM-11 demonstrated greater resistance, with infection rates of 13% and 6% and only mild disease severity. These findings indicated that Mung NM-11 was the most resistant variety, while Mung NM-16 and Abbas Mung were the most vulnerable to fungal infections (Table 1).

Table 1. Percentage of seed infection in six mung bean varieties.

| Sr. No. | Variety | Total no. of seeds tested | Total infection percentage (%) | No. of fungal-infected seeds | Average disease severity |
|---------|--------------|---------------------------|--------------------------------|------------------------------|--------------------------|
| 1 | Mung NM-21 | 15 | 20% | 3 | Moderate |
| 2 | Abbas Mung | 15 | 46% | 7 | Severe |
| 3 | Jumbo Mung | 15 | 13% | 2 | Mild |
| 4 | Mung NM-16 | 15 | 53% | 8 | Severe |
| 5 | Mung NM-11 | 15 | 6% | 1 | Mild |
| 6 | Azri Mung 21 | 15 | 33% | 5 | Moderate |

Purification of Fungal Pathogens: Fungal pathogens isolated from mung bean seeds were purified using PDA medium. Morphological analysis confirmed that *Fusarium* species were predominant in the NM-16 variety, while *T.*

harzianum was isolated from the Jumbo mung variety. This corresponds with the higher seed infection rate observed in NM-16, indicating its greater susceptibility to *Fusarium* (Figure 1).

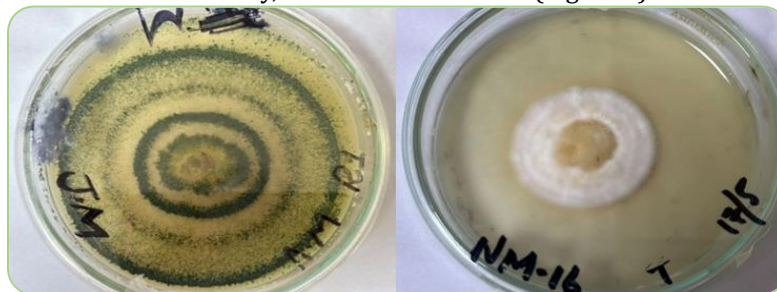


Figure 1. Cultures of *Trichoderma* spp. (left) and *Fusarium* spp. (right) grown on PDA plates.

Microscopic Examination: After seven days of fungal sporulation, spore slides were prepared for microscopic examination. Spores from the NM-16

isolate were observed under a microscope, confirming the presence of *Fusarium* species (Figure 2).

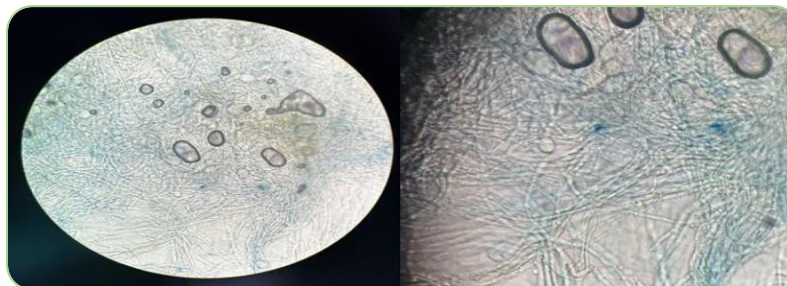


Figure 2. Microscopic view of fungal hyphae and spores.

Evaluation of Treatment Effects on Mung Bean Growth and Disease Management:

The study evaluated the impact of various treatments on mung bean growth and disease management by analyzing different parameters, including the number of leaves, plant height, plant weight, root weight, disease incidence, and disease severity.

Number of Leaves: Table 2 presents the effect of treatments on the number of leaves in mung bean plants. The data were analyzed by comparing the positive control (PC) and negative control (NC). In general, the PC exhibited the expected positive results, while the NC showed no significant effect across all parameters. The highest number of leaves was recorded in the BSD treatment (15.0 leaves), followed by TrH (14.0 leaves). BPM and Fungicide (FD) treatments recorded the lowest average number of leaves (12.0 each). The superior performance of the PC is likely attributed to the inclusion of biochar and biocontrol agents, which are known to promote plant growth by improving soil quality and reducing disease incidence. Similar benefits have been reported by Kizito *et al.* (2019) and Altieri and Nicholls (2020).

Plant Height: Plant height was highest in the BSD treatment (38.0 cm), followed by BTD (35.0 cm), TrH (34.0 cm), and BaS (33.0 cm). The lowest plant height was observed in BPM (32.0 cm), with the fungicide treatment recording the minimum value (31.0 cm), as shown in Table 2. Lehmann and Joseph (2015) reported that biochar enhances plant height by increasing soil nutrient availability and improving water retention. Similarly, Harman *et al.* (2021) noted that biocontrol agents can promote plant growth by enhancing root development and nutrient uptake.

Plant Weight: As shown in Table 2, the highest plant weight was recorded in the BSD treatment (23.0 g), followed by TrH (20.5 g). The lowest plant weight was observed in BPM (18.0 g), while FD had the lowest overall value (16.90 g). Lehmann

et al. (2011) demonstrated significant increases in plant biomass with the application of biochar. Furthermore, Harman *et al.* (2021) showed that biocontrol agents support plant growth by improving nutrient absorption and protecting plants from pathogens.

Root Weight: The effects of different treatments on root weight are presented in Table 2. BSD recorded the highest root weight (5.0 g), followed by TrH (4.6 g). The lowest root weight was found in BPM (4.0 g), with FD again recording the lowest value (3.80 g). These results are consistent with findings by Lehmann *et al.* (2011), who reported improved root biomass with biochar applications.

Disease Incidence: The effectiveness of different treatments on disease incidence is shown in Table 2. The highest disease incidence was observed in BMP (72.20%), followed by BTD (67.20%). The lowest incidence was recorded in TrH (43.60%), with FD showing the least disease incidence overall (23.60%). These results align with studies demonstrate that biochar can reduce disease incidence by enhancing soil health (Lehmann *et al.*, 2011), while biocontrol agents are effective in suppressing plant pathogens (Harman *et al.*, 2021).

Disease Severity (DS): Disease severity was assessed using a 0-9 scale. The highest DS was recorded in BMP (6.20), followed by BTD (5.20). The lowest DS was observed in TrH (3.20), with FD achieving the minimum value (2.20). This research highlights the potential of biochar to improve critical soil conditions, such as nutrient availability and moisture retention that support vegetative plant growth. Moreover, biocontrol agents contribute to plant health by fostering beneficial microbial activity, thereby improving plant development and disease resistance.

Table 2. Effect of different treatments on mung bean growth and disease management.

| Treatments | No. of Leaves | Plant Height (cm) | Plant Weight (g) | Root Weight (g) | Disease incidence (%) | Disease Severity (%) |
|------------------------------------|-------------------------|-------------------------|--------------------------|------------------------|-------------------------|------------------------|
| Biochar Potting Mix (BPM) | 12.0±0.71 ^b | 32.0±0.71 ^b | 18.0±0.35 ^b | 4.0±0.14 ^b | 72.20±1.92 ^a | 6.20±0.83 ^b |
| Biochar Top Dressing (BTD) | 13.0±0.71 ^c | 35.0±0.71 ^c | 20.0±0.35 ^c | 4.50±0.14 ^c | 67.20±1.92 ^a | 5.20±0.83 ^b |
| Biochar Soil Drench (BSD) | 15.0±0.71 ^c | 38.0±0.71 ^{cd} | 23.5±0.35 ^{cd} | 5.0±0.14 ^c | 62.20±1.92 ^a | 4.20±0.83 ^b |
| Fungicide (Difenoconazole) (FD) | 12.0±0.71 ^b | 31.0±0.71 ^b | 16.90±0.54 ^{bc} | 3.80±0.14 ^b | 23.60±4.03 ^b | 2.20±0.83 ^c |
| <i>Trichoderma harzianum</i> (TrH) | 14.0±0.71 ^c | 34.0±0.71 ^c | 20.5±0.35 ^c | 4.60±0.14 ^c | 43.60±4.03 ^b | 3.20±0.83 ^c |
| <i>Bacillus subtilis</i> (BaS) | 13.20±0.44 ^c | 33.20±0.44 ^c | 19.0±0.35 ^{bc} | 4.30±0.14 ^c | 52.20±1.92 ^b | 4.80±0.44 ^b |
| Positive Control (PC) | 16.0±0.71 ^d | 40.0±0.71 ^d | 25.10±0.54 ^d | 5.50±0.14 ^d | 14.60±1.92 ^c | 1.20±0.75 ^c |
| Negative Control (NC) | 8.0±0.71 ^a | 25.0±0.71 ^a | 12.0±0.35 ^a | 2.0±0.14 ^a | 80.40±1.14 ^a | 8.60±0.54 ^a |
| Mean | 12.90±0.67 | 33.53±0.67 | 19.38±0.39 | 4.21±0.14 | 52.00±2.35 | 4.45±0.73 |

CONCLUSION

The present study demonstrates the effectiveness of combining biochar and biocontrol agents in improving mung bean growth and managing diseases. Among all treatments, the fungicide showed the highest efficacy, resulting in the best growth parameters and the lowest disease incidence (23.6%) and severity (2.2%). In contrast, the negative control recorded the poorest performance, highlighting the benefits of targeted treatments. Biochar Soil Drench produced the best vegetative growth, including the highest number of leaves (15), plant height (38 cm), plant weight (23.5 g), and root weight (5 g). *Trichoderma harzianum* also performed well, ranking second in most growth parameters and showing effective disease suppression (DI: 43.6%, DS: 3.2%). *Bacillus subtilis* and Biochar Top Dressing showed moderate results, while Biochar Potting Mix and the fungicide treatment had lower growth values but differed significantly in disease control. Disease incidence and severity were lowest in the fungicide and TrH treatments, confirming their strong biocontrol potential. In contrast, BPM had the highest disease levels (DI: 72.2%, DS: 6.2%), indicating limited effectiveness.

Overall, *T. harzianum* proved most effective for enhancing vegetative growth, while the fungicide achieved the best disease control. The combined use of biocontrol agents and biochar presents a sustainable alternative to chemical treatments.

Future research should explore the mechanisms behind these effects, evaluate long-term impacts, and test these strategies across different mung bean varieties and pathogens.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution of Authors:

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|-------------------|---|
| Tehreem Eraf | : Conducted research activity at the University Research Farm URF, Koont. |
| Tariq Mukhtar | : Conceptualized the idea for the study and supervised the research. |
| Gulshan Irshad | : Provided support at the URF. |
| Muhammad U. Raja | : Designed the methodology and guided in analyzing the data. |
| Javaid Asad | : Provided intellectual input to improve the research article. |
| Syed Zulfiqar Ali | : Helped in data collection. |
| Amar Mehmood | : Helped in data collection. |
| Kachu Z. Haider | : Helped in data collection. |