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"INTEGRATED USE OF BIOCHAR AND BIOCONTROL AGENTS FOR SUSTAINABLE MANAGEMENT OF MUNG BEAN LEAF SPOT DISEASES"

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Abstract

The integrated use of biochar and biocontrol agents for sustainable management of leaf spot diseases in mungbean (Vigna radiata), a vital pulse crop in Asia. Field and laboratory experiments were conducted to evaluate disease resistance across six mungbean varieties and assess the efficacy of treatments including biochar (from cannabis leaves), Trichoderma harzianum, Bacillus subtilis, and fungicides. Results revealed that NM-16 was the most susceptible to leaf spot pathogens, particularly Fusarium spp., while Azri Mung and NM-21 showed moderate resistance. In vitro and in vivo trials demonstrated that biochar and biocontrol agents significantly reduced disease incidence and severity, improved plant growth parameters, and enhanced soil health. The combination of biochar and beneficial microbes proved especially effective, offering an eco-friendly alternative to chemical fungicides. Microscopic and morphological analyses confirmed pathogen identity, and statistical evaluations validated treatment efficacy. The findings underscore the potential of biochar and biocontrol agents in integrated disease management strategies. Future research should focus on optimizing biochar formulations, exploring synergistic effects with other microbial consortia, and scaling up field applications. This approach aligns with global efforts toward sustainable agriculture, offering promising solutions for climate-resilient crop production and reduced reliance on synthetic inputs.

1. INTRODUCTION

Mung bean, *Vigna radiate* is a significant pulse crop in Asian agriculture, mostly in India, Southeast, and East Asia, wherever this one-stop diet based on cereals through a general part of expendable protein via usage using a pulse (Lambrides & Godwin, 2007). Pakistan's pulse production is low due to a lack of innovation, poor seed systems, and various stresses like drought and pests. This forces reliance on imports. To boost production and meet national needs, opportunities exist in developing better crop varieties, adopting intercropping and conservation agriculture, improving certified seed distribution, and providing specific farm machinery and technologies (Ullah et al., 2020). Mung bean is an important and versatile pulse crop, used for food, fodder, and green manure. Despite its importance, production is hindered by low yields due to limited access to improved seeds, low use of modern

practices, and a lack of effective fertilizers. The value chain is also inefficient due to many intermediaries. To improve production, farmers need support with better seeds, modern agronomic techniques, and effective pest control (Dikr, 2023).

2. Climate change threatens pulse crop security, with production lagging behind demand. Intercropping and other innovative systems enhance soil health, biodiversity, and nitrogen fixation. Pulses offer economic benefits and are easy for women to cultivate. The northern Great Plains' warming climate and variable rainfall pose challenges, but adaptive strategies like crop sequencing and altering microclimates can improve pulse resilience and yield despite unpredictable weather extremes (Khan et al., 2025). Study at Layyah, Pakistan, optimized NPK fertilizer for sesame yield under dry conditions. Applying NPK (113:113:60) kg ha^{-1} significantly boosted sesame grain yield (up to 828.6 kg ha^{-1}) for variety TH-6. This rate proves an effective strategy for maximizing sesame productivity despite challenging agro-climatic conditions (Hanif et al., 2024). To combat climate change impacts on agriculture, the Krik house Trust's STOL program focuses on stress-tolerant legume species. These "orphan legumes," like pigeon pea and moth bean, can help smallholder farmers cope with heat and drought. They offer multiple benefits: food, animal fodder, and soil improvement. The program aims to systematically study these neglected crops to restore productivity in climate-stressed regions (Mall, 2017).
3. A study on mung bean disease management found that Cercospora Leaf Spot, caused by *Cercospora canescens*, can be devastating, causing up to 95% yield loss. Of twenty varieties screened, only one (*16051*) showed resistance. In lab tests, the fungicide Score at 100 ppm was the most effective, inhibiting the fungus by 93.03%. The study suggests using line 16051 in breeding programs and testing Score further in field trials (Abbas et al., 2020). A study on spinach grown in cadmium-contaminated soil found that a combination of biochar and compost significantly improved plant growth. The amendments boosted root and shoot length by nearly 50% and 37%, respectively, while also increasing biomass by 43%. This treatment also reduced cadmium content in the roots and shoots by over 40% and 51%, offering a promising solution for soil remediation (Waqas et al., 2024).
4. In a study on country bean, seed-borne fungi were identified, including *Aspergillus niger* and *Rhizopus stolonifer*. Seeds were treated with chemical fungicides and plant extracts. Autostin 50WP was most effective, reducing disease incidence to 33.3% and severity to 21.8%. This led to the highest yield of 5.9 t ha-1, proving its efficacy in controlling diseases and boosting yield (DEY, 2016). study compared enzymatic activities and functional compounds in mungbean pods, stems, and seeds to understand the host-pathogen relationship of Cercospora leaf spot. Resistant genotypes showed significantly higher activity of defense enzymes like ascorbate peroxidase, peroxidase, and catalase in pods after inoculation. These findings highlight that a robust, multi-component antioxidative defense system is crucial for a plant's resilience against this disease (Irfan et al., 2024).
5. A study in Pakistan found that biochar made from Populus leaves, combined with nitrogen and phosphorus fertilizers, significantly improved soil health. Biochar with phosphorus increased microbial biomass carbon by 65% and alkaline phosphatase activity by 24%. Biochar with nitrogen boosted microbial biomass nitrogen by 38% and urease activity by 4%. The study suggests this biochar can enhance soil microbial activity and crop growth (Akmal et al., 2019). A study investigated the use of Enterobacter sp. MN17 and sugarcane bagasse biochar to remediate diesel-contaminated soil. The combined application of these two treatments significantly improved mungbean plant growth, increasing various parameters like shoot and root length and biomass by over 50%. This approach also effectively removed up to 85% of petroleum hydrocarbons from the soil (Ali et al., 2020). A study on cotton found that biochar made from organic waste significantly reduced Fusarium wilt disease. The 9% biochar treatment was most effective, increasing beneficial microbes like Pseudomonas and Trichoderma, while also boosting plant growth, chlorophyll content, and NPK levels. The findings suggest that biochar enhances plant performance by altering the soil microbiome and improving plant defenses (Asif et al., 2023). Global food security hinges on boosting crop yields to meet the 44%

projected demand by 2050, despite severe abiotic and biotic stressors. Healthy soil is crucial, contributing up to 60% of crop yield through improved water retention and nutrient cycling. Addressing challenges demands a holistic approach. Sustainable practices like cover cropping and reduced tillage, combined with precision agriculture technologies such as remote sensing, are essential. This integrated strategy builds resilient agricultural systems to feed the growing population and protect the environment (Hayat, Khan, Klutse, et al., 2025).

6. Agricultural methods have shifted from traditional to conventional, using synthetic fertilizers like the Haber-Bosch process to increase yields, raising environmental concerns. The data highlights issues such as soil degradation, water pollution, and health risks from pesticides. It also notes challenges in animal husbandry, aquaculture, and forestry, underscoring the need for sustainable practices to balance food production with resource conservation (Hayat, Khan, Azam, et al., 2025). A study on lupin plants showed that biochar's effects on growth and disease resistance vary by type. Hydrochar (HTC) from maize silage at a 2% concentration significantly increased root and shoot dry weight by up to 75% and 25%, respectively, and prevented disease. In contrast, wood (WBC) and maize (MBC) pyrolysis chars did not consistently improve growth and sometimes showed disease symptoms (Egamberdieva et al., 2020). A study on mungbean found that a combination of boric acid, ZnSO4, and MgSO4 was the most effective treatment for controlling Cercospora leaf spot disease, resulting in a disease severity of only 11.72%. The research also showed a positive correlation between disease severity and environmental factors like relative humidity, wind speed, and rainfall, while a negative correlation was observed with maximum temperature (Ilyas et al., 2023).
7. A field study in Pakistan found that applying biochar at 20 t ha^{-1} significantly improved mungbean yield and soil health. This treatment, combined with half-levels of phosphorus and potassium, increased 1000-grain weight by 20%, biological yield by 33%, and nodule count by 21%. Additionally, a biochar rate of 40 t ha^{-1} boosted soil nitrogen by 200% and soil organic matter by 94%, demonstrating biochar's effectiveness (Khan et al., 2020). Biochar and compost, when combined, significantly mitigated Cadmium (Cd) toxicity and enhanced Spinacia oleracea growth in contaminated soil. The combined treatment increased root and shoot lengths (49.75%, 36.73%) and biomass (42.85%), while also substantially reducing Cd content in roots (40.41%) and shoots (51.16%). This offers a promising strategy for managing Cd-polluted agricultural soils (Waqas et al., 2024).
8. The use of plant-growth-promoting fungi (PGPF), specifically Trichoderma spp. and Penicillium spp., in agriculture and horticulture. The review, based on various studies, found that these fungi are effective, affordable, and eco-friendly biocontrol agents. They enhance plant growth by improving nutrient absorption, boosting morphological growth, increasing yield, and inducing disease resistance, ultimately contributing to sustainable crop production (Sun et al., 2025). study screened 31 cotton varieties against bacterial blight. Five varieties (FH-142, FH-326, FH-Kehkashan, FH-468, FH-152) were resistant. Chemically, Flare-72 SP (Streptomycin Sulphate) was the most effective treatment. Among plant extracts, *Azadirachta indica* (Neem) at 15% concentration showed the best bacterial inhibition and significantly improved plant growth parameters (Zia et al., 2023).
9. A study on Charcoal rot in green and black gram found that seven *Trichoderma* species were effective against the pathogen *Macrophomina phaseolina*. *T. harzianum* showed the highest efficacy, reducing fungal growth by nearly 80%, minimizing sclerotia size, and increasing plant germination (86.67%) and survival. This suggests *Trichoderma* is a promising, natural alternative to fungicides for managing the disease (Iqbal & Mukhtar, 2020). The *Bacillus cereus* group produces diverse antimicrobial compounds that have broad applications. These bacteria act as effective biocontrol agents against plant pathogens and nematodes, and they aid in bioremediation by degrading pollutants. They are also used in industry for creating biofertilizers and nanoparticles. While some strains can cause food poisoning, their potential for sustainable solutions in agriculture, medicine, and environmental cleanup is significant (Azizoglu et al., 2025).

A study on tomato plants found that green waste biochar (GWB) combined with the bacterium *Bacillus subtilis* was

most effective at suppressing early blight disease. This treatment reduced the disease by up to 80% and significantly improved plant growth and physiology. The research highlights the synergistic effect of GWB and *B. subtilis* in managing foliar pathogens and enhancing plant health (Rasool et al., 2021). Biochar is an eco-friendly alternative to chemical treatments for plant diseases. By improving soil's chemical, physical, and biological properties—such as its acidic pH and porous structure—it enhances soil health. This, in turn, boosts beneficial microorganisms and plant resistance, effectively controlling soil-borne pathogens and promoting healthier plant growth (Hou et al., 2022). A review of 12 studies found that biochar is highly effective at suppressing plant diseases, with 85% of cases showing a suppressive effect against both soilborne and airborne pathogens. The mechanisms include inducing plant resistance, enhancing beneficial microbes, and modifying soil quality. However, more research is needed to fully understand the long-term effects and widespread application of biochar (Bonanomi et al., 2015).

MATERIALS AND METHODS

The varieties were collected from the National Agriculture Research Center (NARC). The trial was laid out in a Randomized Complete Block Design (RCBD) with 8 repetitions. The crop was harvested on 26th October. In the experiment, standard agricultural practices were followed consistently, with

In the current instruction, research laboratory work, with isolations, morphologic classification, Purification, Spores Slide Preparation, In Vitro Evaluation of Biochar and Biocontrol Agents Against seed borne Pathogens of Mungbean and In-vivo Screening of Mungbean Germplasm Against Leaf Spots Under Field Conditions were performed at the Fungal Plant Pathology Laboratory.

In vivo screening of mungbean germplasm against leaf spots under field conditions:

The research was conducted at the University Research Farm URF Koont to investigate the basis of resistance and susceptibility in contradiction to mungbean leaf spot disease. The experiment employed 6 different varieties of mungbean (AZRI Mung 2021, Mung NM-1, JUMBU Mung, Mung NM-16, Abbas Mung, Mung 2021) seeds acquired from the NARC National Agriculture Research Council department of CSI Crop Sciences Institute from Pulses Program with a seed rate of 20kg/ha. The design size was 3m × 4m and seeds remained spread on 3rd August.

treatments categorized into treated and untreatedgroups to evaluate their impact on disease management. Insecticides were deliberately withheld to promote natural disease spread. At the reproductive stage, 15 plants from each plot were randomly selected and assessed using a 0–9 rating scale.



Figure 1: Mungbean germplasm sowing for in vivo screening under field conditions.

Assessment of Disease Incidence and Severity in Cultivars Using Disease Rating Scale:

Disease incidence and severity were monitored weekly. The cultivars were evaluated using a specific rating scale. The frequency of disease events was recorded regularly, and the percentage of disease incidence was determined using a standard calculation formula;

$$\textbf{Percentage Disease Incidence (PDI)} = \frac{\textit{number of plants infected in a row}}{\textit{total number of plants in a row}} \textit{ x } 100$$

The genotypes were evaluated using a scale from 0 to 9, with 0 indicating complete immunity to the disease and 9 representing extreme susceptibility. This scale was used to determine and classify the degree of disease severity across different genotypes.

Table 1: Disease Rating Scale

Disease Rating/Grade	Percent Leaf Area Affected	Description	Disease Response
0	-	No infection	Immune
1	1-10	One spot to 10% diseased area	Resistant(R)
3	11-30	11-30% diseased area	MR
5	31-50	31-50% diseased area	MS
7	51-70	51-70% diseased area	Susceptible
9	>71	>71% diseased area	HS

In vitro evaluation of biochar and biocontrol agents against leafspot pathogens of mungbean:

Mungbean (Vigna radiata) cultivation is greatly impacted by leaf spot diseases, which makes appropriate disease management techniques necessary. The efficiency of biochar and biocontrol agents against these pathogens in vitro is assessed in this study. The capability of biocontrol agents to suppress pathogens in an ecologically friendly method and the soil-enhancing qualities of biochar present appealing approaches to sustainable disease management in mungbean.

Isolation and Purification of fungal leaf spot pathogens in mungbean varieties:

To detect fungal pathogens on mungbean leaves, we gathered parts of the leaves showing symptoms and processed them under sterile conditions. Initially, we used a sterile scalpel to remove the infected areas of the leaves, ensuring that only the affected regions were sampled. The sections were then purified by drenching them in a 2% Clorox resolution for one minute to disinfect their surfaces. Following that, the leaf sections were rinsed in sterile distilled water for 30 seconds to eliminate any remaining Clorox, and this rinsing process was repeated in a second plate of sterile distilled water for thorough cleansing.

After sterilization, the leaf sections were placed on sterilized filter papers to air dry. Once dry, approximately small sections were cut and placed onto Potato Dextrose Agar (PDA) media in purified petri plates. These plates were wrapped with parafilm, properly labeled, and then raised at 25°C in the dark for 5-7 days. We conducted regular checks on the plates to observe the growth of fungal colonies, which were then transferred to fresh PDA plates using a sterile loop or needle to obtain pure cultures. The morphological characteristics of the isolated fungi were examined under a microscope, and additional biochemical or molecular tests were performed as necessary to accurately identify the fungal species.

Identification and Morphological Characterization

To begin with, symptomatic leaf sections were gathered and surface sterilized to avoid any contamination. After sterilization, the leaf segments were air-dried and placed on Potato Dextrose Agar (PDA) media inside sterile petri dishes. The dishes were then kept in the dark at 25°C for 5-7 days to allow fungal colonies to develop. Once colonies appeared, they were transferred to new PDA plates to isolate pure cultures.

Afterward, we prepared a slide for detailed observation. Following 7 days of fungal sporulation, we took a clean glass slide, placed a drop of blue lactophenol in the centre under a laminar flow hood, and gently scraped spores from the fungal fruiting bodies using a sterile needle, transferring them into the drop of blue lactophenol on the slide. A coverslip was carefully placed over the spores and gently pressed down to spread them evenly. The slide was then allowed to air dry completely before being placed on the microscope stage.

Upon microscopic examination, we observed characteristic Alternaria hyphae for the mungbean variety NM-16, but no spores were found, prompting a shift in focus to seed pathology for further investigation into fungal presence and behaviour.

Identification and Isolation of Seed-Borne Pathogens in Mungbean Varieties

In seed pathology, after autoclaving the distilled water and filter papers, allow the distilled water to cool down at room temperature before filling three sterilized petri plates with 2% Clorox and the remaining petri plate with distilled water. Collect the seeds of each mungbean variety. Divide the seeds into three groups for each variety. Take two plates for each variety and sterilize them by wiping them with a cloth soaked in 2% Clorox solution. Make sure to rinse the plates with distilled water afterward.

Place the sterilized plates in a controlled and clean environment in laminar flow. With sterile forceps put the seeds on a Petri plate filled with 2% Clorox for 1 minute and then another Petri plate filled with distilled water for 30 seconds. After sterilizing the seeds, place them on sterilized filter paper to dry. When the seeds have fully dried place them on PDA media using forceps. Close the plates and seal them with parafilm to prevent contamination. Label the Petri plate with a variety of names and dates. Incubate the plates under appropriate conditions for the mungbean seeds to germinate and grow at 25°C. Observe the plates regularly for any signs of seed-borne pathogens, such as fungal growth or discoloration. Document and compare the results for each variety and sterilization method. They recognized the definite seed-borne pathogens distressing mungbean in different regions. Isolated and detected the pathogens that cause infections, as well as created the inoculum.

Evaluation of the Percentage of Seed Infection in Different Varieties of Mung beans:

The proportion of seed infection is a significant influence in evaluating the health and viability of mungbean (Vigna radiata) seeds. This research assessed seed-borne pathogen infection on Potato Dextrose Agar (PDA) medium for six mungbean varieties. The objective is to identify the susceptibility of each variety to pathogens and offer insights for developing effective disease management strategies.

Formula:

$$\textbf{Seed infection percentage} = \frac{\textbf{No. of infected seeds}}{\textbf{Total no. of seeds}} \; x\,100$$

Purification of Fungal Pathogens from Culture Media

Prepare the PDA according to the instructions on the packaging. This typically involves dissolving the necessary quantity of PDA powder in distilled water, boiling the mixture until the medium is fully dissolved, and then sterilizing it by autoclaving it for 15 to 20 minutes at 121°C. Once the medium has been autoclaved, transfer it into aseptic, sterile petri dishes and let it cool and solidify before using.

To isolate and purify fungal pathogens, first grow the fungi on an appropriate medium, such as Potato Dextrose Agar (PDA). Look for distinct colonies with different appearances. Using a sterilized scalpel or inoculation loop, transfer a small portion of these colonies to the center of a fresh PDA plate. Incubate this plate at 25–28°C for approximately five days or until you see sufficient fungal growth.

For further purification, use a sterile needle or scalpel to remove a hyphal tip from the newly formed fungal colony and transfer it to a new PDA plate to minimize

contamination. Keep the plate under the same incubation conditions. Repeat this process of transferring hyphal tips to fresh PDA plates until a pure culture is obtained, which typically requires two or three subculturing cycles. Throughout this process, regularly examine the fungal cultures under a microscope to assess their morphological characteristics and ensure they match the known descriptions of the pathogen.

Mass Multiplication

Inoculate pure fungal culture on sterilized petri plates. Incubate plates at optimal temperature for fungal growth. Allow the fungus to grow and form colonies on the agar subculture by transferring a piece of fungal colony to fresh PDA plates. Repeat subculturing to increase fungal biomass.

Microscopic Examination: Spore Slide Preparation

After 7 days when fungal sporulation is formed prepare a slide to identify the pathogen. Take a clean glass slide and place a drop of blue lactophenol in the center under the laminar flow. Using a sterile needle, gently scrape the spores from the fungal fruiting body (Mung NM-16, Jumbo Mung).

Transfer the collected spores into the drop of blue lactophenol on the slide. Place the coverslip over the spores carefully to avoid air bubbles. Gently press down on the coverslip to spread the spores. Allow the slide to air dry completely before observing under a microscope. Placing a prepared slide on the microscope stage. Identify the specimen accurately. (Fusarium from NM-16, Trichoderma Harzianum from Jumbo mung).

Pot Preparation and Seed Sowing Techniques

This research was conducted during the mung bean season at the trial site of University Research Farm URF Koont. This research aims to evaluate the probability of biochar and biocontrol agents in the control and monitoring of leaf spot diseases in mungbean crops. I planted 40 pots across the greenhouse, then selected a susceptible mungbean (NM-16) and placed each variety in 40 pots. I prepared a potting mixture by mixing organic matter, peat moss, soil, and sand in the ratio 1:1:2:1.

Fill each pot with the potting mixture, leaving some space at the top for sowing the seeds. Place 5 Mungbean seeds in each pot. Shelter the seeds with a tinny coating of the potting mixture. Aquatic the pots gently to moisten the soil but avoid overwatering. Place the pots in a sunny location with good air circulation. Then I labeled the pots with a variety of names and treatments. Monitor the moisture levels and water the pots when the top layer of soil feels dry. After reaching the seedling stage of the mungbean. Apply 8 different treatments with 5 replications each. Following this, Mungbean leaves were inoculated with a suspension of the seed-borne pathogens after 48 hours of treatments. Analyze the effectiveness of each treatment on the mungbean pathogen.

Biochar (Cannabis leaves) and biocontrol agents will be applied to the soil. Treatments will be tested with various quantities and combinations to determine the most effective one. Inoculated leaves will be located in an organized environment with suitable temperature, humidity, and light circumstances for pathogen growth.

Monitor and estimate the development and severity of the pathogen in each treatment repeatedly. Parameters such as lesion size, disease incidence, and disease severity should be measured. Statistical analysis will be used to estimate the presentation of dissimilar treatments and determine whether biochar, biocontrol agents, or their combination decreases disease incidence and severity significantly.

RESULTS AND DISCUSSION

In Vivo Screening of Mungbean Germplasm Against Leaf Spots Under Field Conditions:

To assess mungbean germplasm resistance to leaf spots in field conditions, we performed an in-vivo screening. Started by leveling and plowing the experimental plot to make sure the soil was ready for growing mung beans. For the study, we chose a wide range of mungbean germplasm, including cultivars that were known to be resistant to leaf spots and susceptible to them. To guarantee objective outcomes, every variety was planted in randomized plots. We closely watched the plants over the growth season, observing the emergence and development of leaf spots. Evaluated each variety's pathogen severity using a standardized disease rating scale.

Comprehensive observations and documentation of disease symptoms, plant growth, and yield parameters were part of the data-gathering process. In the

experiment, standard agricultural practices were followed consistently, with treatments categorized into treated and untreated groups to evaluate their impact on disease management. Insecticides were deliberately withheld to promote natural disease spread. It was examined that the data gathered throughout the growth season to determine which germplasm showed notable resistance to leaf spots. We were able to identify the most promising mungbean types for upcoming breeding initiatives targeted at improving disease resistance thanks to this thorough assessment. We determined each variety's disease index using the disease rating scale, which gave me a numerical indicator of how resistant they were to various diseases.

Assessment of Disease Incidence and Severity in Cultivars Using Disease Rating Scale:

Disease incidence and severity were assessed weekly. Each cultivar was evaluated using a disease rating scale. Disease incidence was tracked regularly, and the percentage of disease incidence was calculated using a specific formula.

$$\textbf{Percentage Disease Incidence (PDI)} = \frac{\textbf{No. of infected plants in row}}{\textbf{Total no. of plants in row}} \textbf{ x 100}$$

Figure 1: Collection of samples from infected mungbean plants in the field for analysis.



Table 1: Disease incidence and severity of leaf spot in mungbean varieties at Chakwal Koont Farm.

Sr. no	Varieties	Total no of plants (8 row)	Total no of infected plants (8 rows)	Percentage disease incidence %	Disease severity rating	Disease response
1	Mung NM-21	240	57	23.75%	3	Moderately Resistance (MR)
2	Abbas Mung	240	97	40.42%	5	Moderately Susceptible (MS)
3	Jumbo Mung	240	83	34.58%	5	Moderately Susceptible (MS)
4	Mung NM-16	240	117	48.75%	7	Susceptible (S)
5	Mung NM-11	240	87	36.25%	5	Moderately Susceptible (MS)
6	Azri Mung	240	57	23.75%	3	Moderately Resistant (MR)

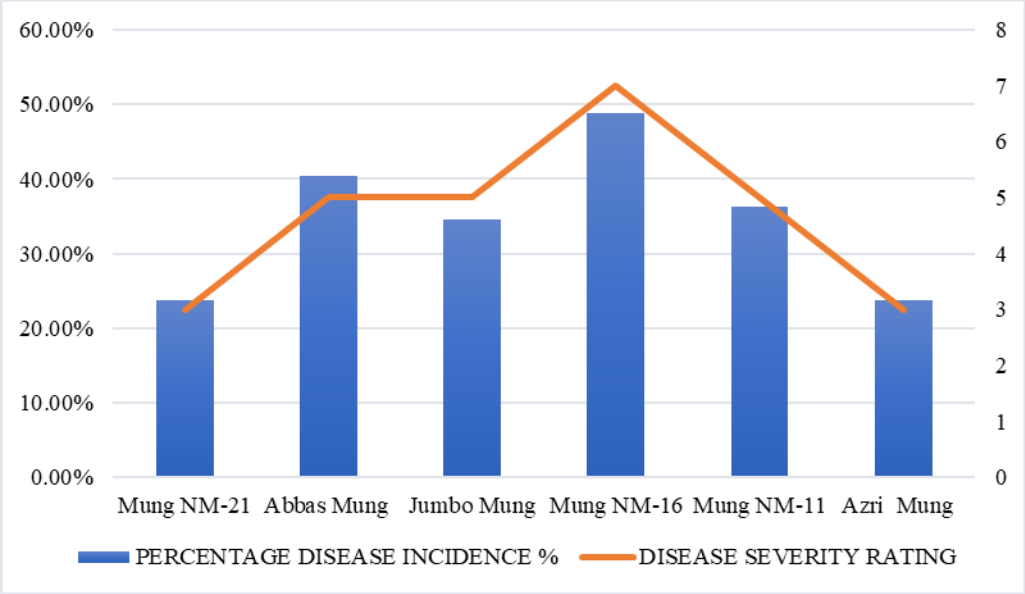


Figure 3: Disease incidence and severity in mung varieties.

Disease Severity Rating based on the percentage of leaf area affected, classified as follows:

0: Immune (I)
1: Resistant (R) (1-10% diseased area)
3: Moderately Resistant (MR) (11-30% diseased area)
5: Moderately Susceptible (MS) (31-50% diseased area)
7: Susceptible (S) (51-70% diseased area)
9: Highly Susceptible (HS) (>71% diseased area)

Mung NM-16 has the highest disease incidence at 48.75%, classified as "Susceptible" with a severity rating of 7.

AZRI Mung 2021, and Mung 2021 show moderate resistance with a severity rating of 3, indicating that 11-30% disease area is affected.

Table 2: Response of Cultivars to Treatments:

Cultivar	Mean
Abbas Mung	7.6483
Azri Mung	4.6083
Jumbo Mung	6.1483
Mung NM-11	7.0033
Mung NM-16	9.5867
Mung NM-21	4.63

The mean responses of different mungbean cultivars to the treatments, with Mung NM-16 exhibiting the highest susceptibility (9.5867) and Azri Mung and Mung NM-21 exhibiting the lowest responses (4.6083 and 4.6300). These results align with prior research, such as Binyamin et al. (2022) and Iqbal et al. (2021), which reported similar trends in disease resistance among mungbean varieties, highlighting the importance of selecting genetically resistant cultivars for effective disease management.

Table 3: Response of Cultivars

Cultivars	Mean
Mung NM-16	9.5867 [a]
Abbas Mung	7.6483 [b]
Mung NM-11	7.0033[bc]
Jumbo Mung	6.1483[c]
Mung NM-21	4.63[d]
Azri Mung	4.608LDS3[d]
LSD 5%	

The mean responses of different mungbean cultivars to the treatments, with statistical significance indicated by letters. Mung NM-16 exhibited the highest mean response (9.5867 a), indicating the greatest susceptibility or reaction to the treatments. In contrast, Azri Mung and Mung NM-21 displayed the lowest responses (4.6083 d and 4.6300 d, respectively), suggesting better resistance. The different letters indicate significant differences between the cultivars, as determined by the Least Significant Difference (LSD) test at the 5% level.

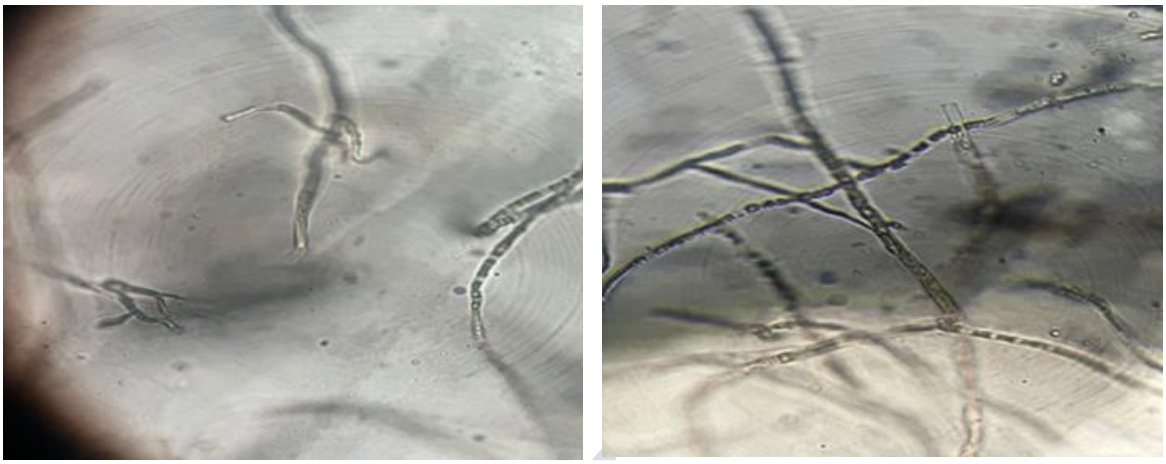
In Vitro Evaluation of Biochar and Biocontrol Agents Against Leafspot Pathogens of Mungbean:
The efficiency of biochar and biocontrol agents against mungbean leaf spot diseases (Vigna radiata) is tested in vitro. By investigating how these treatments can improve plant health and productivity and offer a more environmentally friendly option to chemical pesticides, this study seeks to examine sustainable agriculture methods.

Isolation and Purification of fungal leaf spot pathogens in mungbean varieties:
We collected fungal pathogens from diseased mungbean leaves by cutting out the infected areas and sanitizing them with a 2% Clorox suspension, surveyed by cleaning in disinfected purified water. After the leaf sections had dried, we placed them on Potato Dextrose Agar (PDA) in Petri plates and raised them at 25°C in the dark for 5-7 days.
We observed fungal collections, transferred them to fresh PDA plates for purification, and characterized them based on their physical attributes. Additional biochemical or molecular tests were performed for precise species identification. This method effectively identified the fungal pathogens responsible for leaf spots on mung beans, thus contributing to the

comprehension and control of the disease.

Identification and Morphological Characterization
After surface-sterilizing the symptomatic mungbean leaves, we placed them on Potato Dextrose Agar (PDA) plates and incubated them in the dark at 25°C for 5-7 days. We observed the growth of fungal colonies and then transferred these colonies to fresh PDA plates to purify them. Subsequently, we used blue lactophenol to prepare slides for microscopic examination, revealing characteristic Alternaria hyphae in the mungbean variety NM-16, prompting a shift towards investigating the fungal presence and behavior in seed pathology.

Figure 4: Microscopic view of *Alternaria* hyphae in Mung bean Leaf Samples:



Identification and Isolation of Seed-Borne Pathogens in Mungbean Varieties:
Seed-borne pathogens significantly threaten agriculture, impacting crop germination, strength, and yield. Identifying these diseases is essential for understanding their effects and creating effective control methods. This research concentrates on isolating seed-borne diseases from six types of mungbean (Vigna radiata) using a Potato Dextrose Agar (PDA) media.

The consequences revealed the presence of different diseases in the various mungbean types. Following three days of cultivation on PDA medium, the cultures were purified for further examination. Based on their appearance, Fusarium species were specifically identified from the NM-16 variety at the Fungal Plant Pathology Laboratory. This identification highlights the susceptibility of the NM-16 variety to Fusarium infection, providing critical insight for targeted disease management strategies.

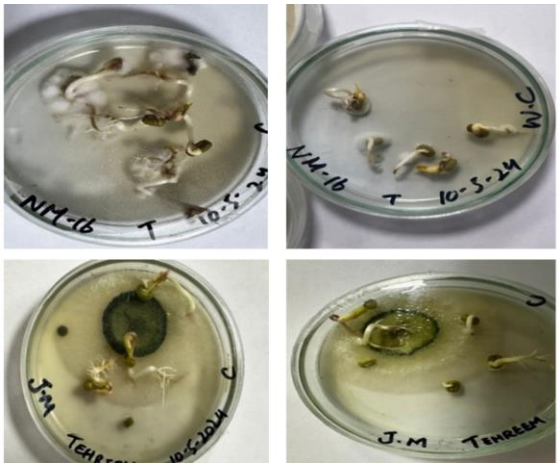


Figure 5: Seedling disease progression on agar plates.

Evaluation of the Percentage of Seed Infection in Different Varieties of Mung beans:

The evaluation of seed infection percentages in different mungbean varieties can be found in Table 3. This analysis offers the equal of seed-borne pathogen infection in each variety. The results indicate the following infection percentages and the number of fungal infections for each variety with the seed infection percentage formula.

$$\textbf{Seed infection percentage } \% = \frac{\textbf{No. of infected Seeds}}{\textbf{Total no. of Seeds}} \textbf{ x 100}$$

1. Mung NM-21: 20% overall infection, with 3 fungal infections
2. Abbas Mung: 46% overall infection, with 7 fungal infections
3. Jumbo Mung: 13% overall infection, with 2 fungal infections
4. Mung NM-16: 53% overall infection, with 8 fungal infections
5. Mung NM-11: 6% overall infection, with 1 fungal infection
6. Azri Mung 21: 33% overall infection, with 5 fungal infections

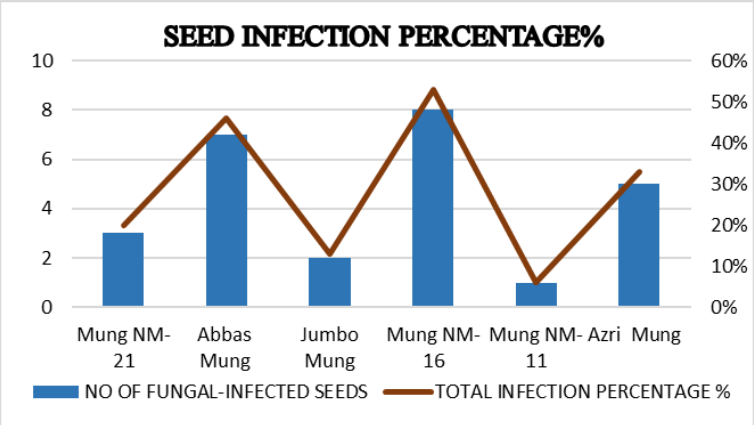
The data indicate that the NM-16 variety is the most susceptible and exhibits the highest total infection percentage of 53%, showing 8 instances of fungal infection. This outcome highlights the increased vulnerability of NM-16 compared to the other varieties examined. In contrast, Mung NM-11 displays the lowest infection percentage at 6%, signifying a greater resilience to seed-borne pathogens.

The higher infection rates observed in NM-16 imply the necessity for targeted disease management practices to address its susceptibility. Further exploration of the contributing factors to its higher susceptibility could yield valuable insights for developing effective control measures.

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

Table 4: Seed Infection Percentage%

Figure 6: Seed Infection Percentage:



Distinct differences in susceptibility to fungal infection were observed in various mungbean varieties during the evaluation. Mung NM-21 and Azri Mung 21 showed moderate resistance, with infection rates of 20% and 33% and moderate disease severity.

Highly susceptible varieties included Abbas Mung and Mung NM-16, with infection rates of 46% and 53% and severe disease severity. Jumbo Mung and Mung NM-11 exhibited mild resistance, with infection rates of 13% and 6% and mild disease severity.

These results suggest that Mung NM-11 is the most resistant variety, while Mung NM-16 and Abbas Mung are the most susceptible to fungal infection.

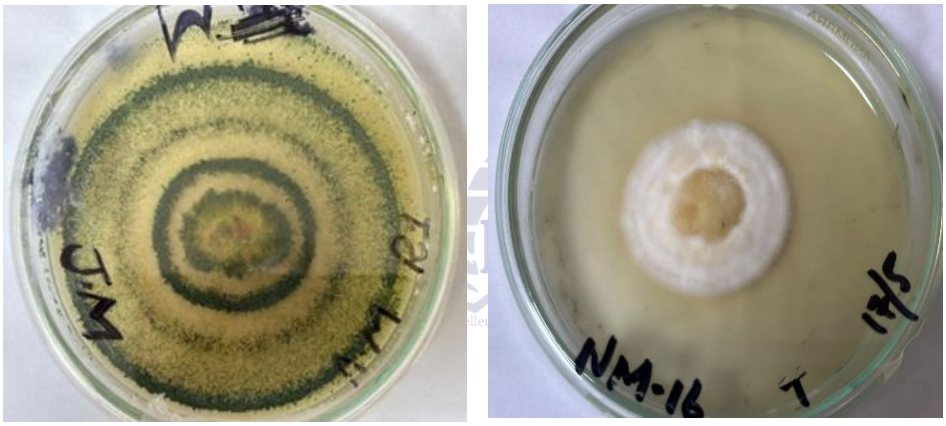
Purification of Fungal Pathogens from Culture Media:

The identification and purification of fungal pathogens from mungbean seeds were conducted to determine the specific pathogens found in different varieties. Following three days of placement on the Potato Dextrose Agar (PDA) medium, the cultures were purified for further examination.

The following steps were implemented to ensure exact identification and characterization:

Mungbean seeds were cultivated on a PDA medium for three days to encourage the growth of any fungal pathogens present. After the incubation period, individual fungal clusters were transported to new PDA plates to attain pure cultures.

Figure 7: Cultures of *Trichoderma spp.* (left) and Fusarium spp. (right) grown on agar plates.



The purified cultures were subjected to morphological characterization in the Fungal Plant Pathology Laboratory. The results revealed that Various fungal pathogens were found in different mungbean varieties during the purification process. NM-16, in particular, was identified to have Fusarium species, indicating its higher susceptibility to this pathogen. This finding corresponds with the data on seed infection percentages, which also pointed to NM-16 having the highest overall infection rate.

The successful purification and identification of Fusarium from NM-16 underscores the need for targeted disease management strategies for this specific variety. Future efforts should concentrate on developing resistant varieties and implementing effective control measures to minimize the impact of seed-borne pathogens in mungbean cultivation.

Mass Multiplication

For detailed studies, it was crucial to mass-multiply fungal pathogens. At first, fungal pathogens were isolated and purified from infected mungbean seeds using a Potato Dextrose Agar (PDA) medium. Following this, large amounts of PDA were prepared, and the purified cultures were inoculated and incubated to grow under optimal conditions.

The resulting cultures were strong and plentiful, suitable for further pathogenicity tests and interaction studies with mungbean seeds. This method efficiently produced the required quantities of pure fungal cultures, making it easier to conduct thorough research on seed-borne pathogens and contributing to improved disease management approaches in mungbean cultivation.

Microscopic Examination: Spore Slide Preparation

Upon the completion of fungal sporulation for seven days, we proceeded to prepare slides to identify the pathogens present in the different mungbean varieties. The preparation process commenced by placing a clean glass slide under a laminar flow and placing a drop of blue lactophenol in the center of the slide. With the use of a sterile needle, spores were accurately scraped from the fungal fruiting bodies of the NM-16 and Jumbo Mung varieties. Subsequently, these spores were carefully transferred into the drop of blue lactophenol on the slide. A coverslip was then carefully positioned over the spores to prevent air bubbles, and gentle pressure was applied to ensure an even spread of the spores.

The slides were left to dry in the open air until they were completely dry, after which they were inspected using a microscope. This approach allowed for accurate identification of the fungal pathogens. Fusarium species from NM-16 and Trichoderma harzianum from Jumbo Mung. The microscopic examination was crucial for confirming the particular fungal pathogens that impacted the mungbean varieties, and it yielded important information for future research and disease control.

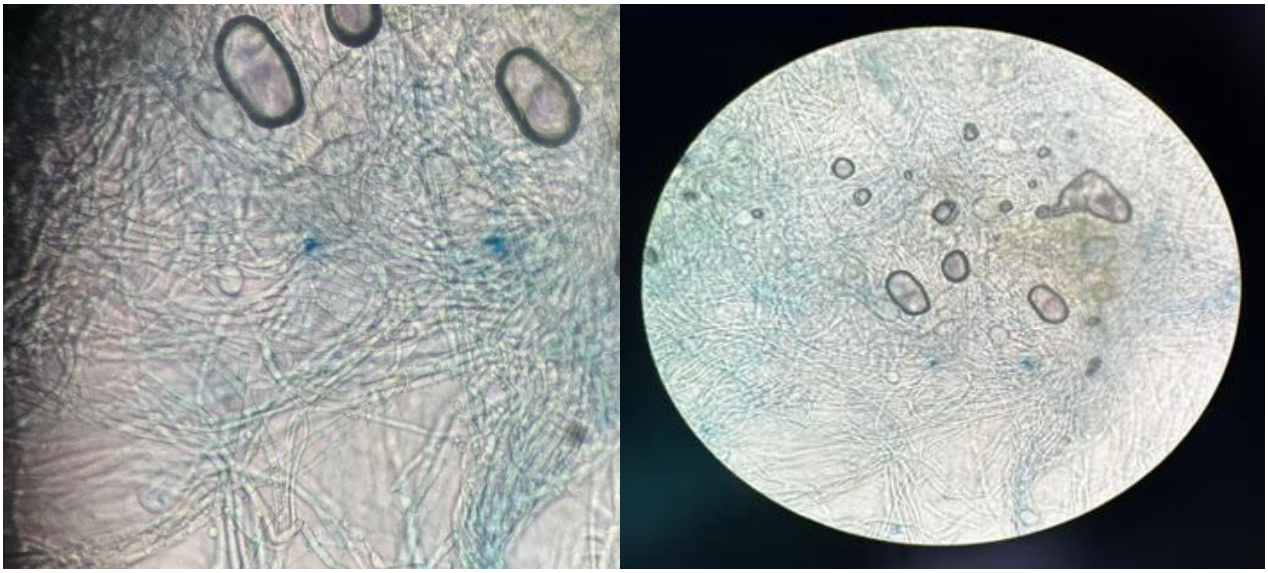


Figure 8: Microscopic view of fungal hyphae and spores

Pot Preparation and Seed Sowing Techniques

The learning expected to evaluate the efficiency of biochar and biocontrol agents in managing and monitoring leaf spot diseases in mungbean crops at URF Koont, the University Research Farm. A total of 40 pots were prepared using a potting mix consisting of organic matter, peat moss, soil, and sand in a 1:1:2:1 ratio. Each pot was filled with the mixture, leaving space for seed sowing. Five seeds of the vulnerable mungbean variety NM-16 were planted in each pot, lightly covered with the potting mix, and gently watered to prevent overwatering. The pots were positioned in a well-lit area with good air circulation.

Following the emergence of the seedlings, eight distinct treatments were utilized, and each action was repetitive five times. The treatments were managed right after sowing, and a pathogen inoculum was applied 48 hours after the treatment. Biochar (Cannabis Leaves) and biocontrol agents were applied based on the treatments, and the progress and severity of leaf spot diseases were assessed under measured environmental conditions to assess the effectiveness of each treatment.

The experiment treatments are:

T1 Positive Control (PC)
T2 Negative Control (NC)
T3 Biochar Potting Mix (BPM)
T4 Biochar Top Dressing (BTD)
T5 Biochar Soil Drench (BSD)
T6 Fungicide (Difenoconazole) (FD)
T7 *Trichoderma harzianum* (TrH)
T8 *Bacillus subtilus* (BaS)

Parameters

This research purposes to assess the impact of several treatments on mungbean growth and disease management by examining key parameters at the plant's maturity stage. Evaluating the number of leaves, plant height, plant weight, root weight disease incidence %, and disease severity is essential for understanding the influence of different treatments, such as biochar, biocontrol agents, and fungicides, on plant health and disease control. Through the analysis of these metrics, the study seeks to determine the most effective strategies for enhancing mungbean cultivation.

At the maturity stage following parameters were recorded: No of Leaves, Plant Height, Plant weight, Root weight, Disease incidence and Disease severity.

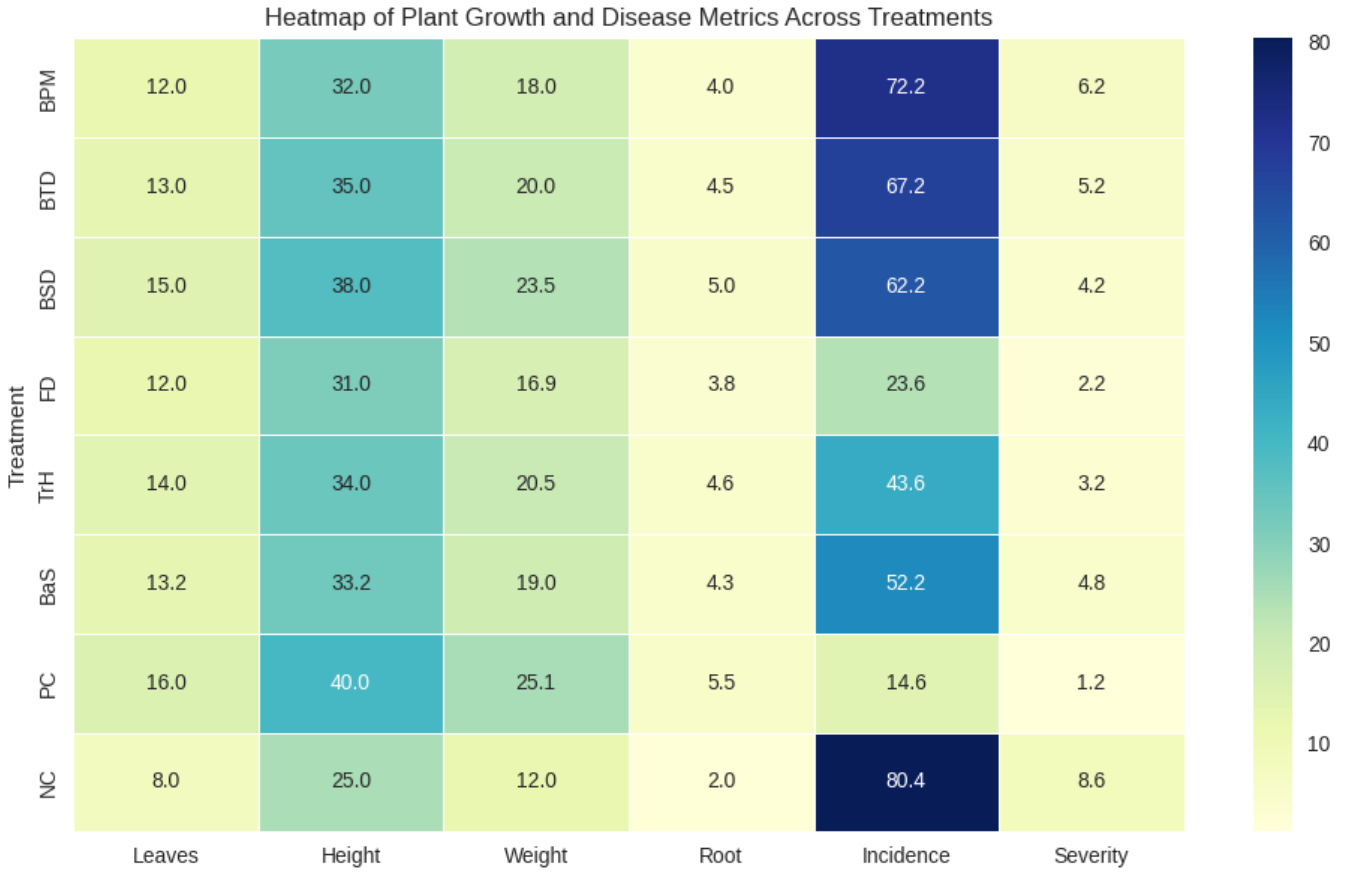


Figure: This heatmap compares plant growth and disease metrics across treatments, highlighting which interventions promote healthier growth or reduce disease. It visually reveals patterns—like high disease incidence in NC—helping and identified with effective treatments and optimize agricultural strategies.

Table 2 Effect of different treatments on number of leaves as a growth parameter in mung beans

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}
Trichoderma harzianum (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}
Bacillus subtilus (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±1.14a	8.60±0.54a
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**

Conclusions:

In response to the increasing threat of leaf spot diseases in mungbean fields, this study investigates the effectiveness of biochar and biocontrol agents as sustainable approaches for disease management. The research included two main experiments: a controlled pot experiment and a field trial. The investigation started with a comprehensive screening of mungbean varieties to identify possible fungal pathogens. This process led to the identification of Alternaria hyphae in the infected leaves. However, despite the presence of hyphae, no spores were observed, which is critical since spore formation is necessary for pathogen inoculum production. Particularly, the Alternaria isolates did not progress to the sporulation stage even under optimal laboratory conditions, which further complicated the development of an effective inoculum. This inability of Alternaria to progress to the sporulation stage, which is essential for producing viable inoculum, prompted a shift in focus towards seed pathology to further investigate and identify the underlying fungal pathogens.

The controlled pot experiment, carried out at the URF Koont, University Research Farm, involved the preparation of 40 pots containing a potting mix of organic matter, peat moss, soil, and sand. Each pot was planted with five seeds of the susceptible mungbean variety NM-16. Subsequently, treatments comprising biochar and biocontrol agents were applied post-sowing, followed by disease inoculation. Various growth and disease parameters such as leaf count, plant height, plant weight, root weight, disease incidence, and severity were evaluated using statistical analyses such as ANOVA and LSD tests to gauge the efficacy of the treatments. The findings indicate that treatments incorporating biochar and biocontrol agents were effective in managing Fusarium, with the biochar treatment proving to be particularly effective. This offers a promising alternative to conventional controls and chemical fungicides, providing a step forward in disease management.

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