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RESEARCH PAPER

# Evaluation of Trichoderma-Based Bio-Fungicide and Poultry Manure for the Management of Foot and Root Rot Disease in Mung Bean (*Vigna radiata*) Caused by *Sclerotium rolfsii*

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**ABSTRACT**

Foot and root rot disease, caused by *Sclerotium rolfsii*, poses a major constraint to mung bean (*Vigna radiata*) production, leading to significant yield losses. This study aimed to evaluate the efficacy of *Trichoderma*-based bio-fungicides and organic amendments, particularly poultry manure, in managing the disease and enhancing mung bean growth and yield under field conditions. Additionally, the antagonistic potential of *Trichoderma* formulations against *S. rolfsii* was assessed in vitro. Field experiments were conducted in a naturally infested field using a Randomized Complete Block Design (RCBD) with four replications. Six organic amendments—poultry manure, cow dung, wood ash, vermicompost, mustard oil cake, and sunflower oil cake—were tested alongside seed treatment with BAU-Biofungicide (*Trichoderma harzianum*). Pathogen isolation and identification were performed, and the antagonistic potential of *Trichoderma* formulations was evaluated using the dual culture method. Field emergence, pre- and post-emergence mortality, and yield parameters were recorded and analyzed using ANOVA and LSD tests at $p<0.05$. In vitro analysis revealed that BAU-Biofungicide and *Trichoderma* suspension significantly inhibited *S. rolfsii* mycelial growth by 71.11% and 65.83%, respectively. Field trials demonstrated that poultry manure and vermicompost significantly improved field emergence (75.0% and 70.0%, respectively) and reduced pre- and post-emergence mortality compared to the control. Poultry manure resulted in the highest grain yield (922.5 kg/ha), followed by vermicompost (917.5 kg/ha), with yield increases of 29.93% and 29.23% over the control, respectively. This study highlights the effectiveness of *Trichoderma*-based bio-fungicides, particularly BAU-Biofungicide, and organic amendments such as poultry manure and vermicompost in managing *S. rolfsii* and enhancing mung bean productivity. Integrating biological control agents with organic amendments presents a promising strategy for sustainable disease management and improved crop yields. Future research should focus on optimizing application methods and assessing long-term soil health benefits.

**Key words:** Foot and root rot disease, Organic amendments, *Sclerotium rolfsii*, Mung bean

## Introduction

Mung bean (*Vigna radiata*), commonly known as green gram, is a widely cultivated legume native to Southeast Asia. It is an important protein-rich food source and is extensively used for both human consumption and livestock feed. Additionally, mung bean plays a crucial role in sustainable agriculture by enriching soil nitrogen, particularly in crop rotation systems with rice. In Bangladesh, it is cultivated on approximately 44.25 thousand hectares, with an annual production of 41.19 thousand tons and an average yield of 93.08 tons per hectare. Its cultivation has been expanding due to its short growth cycle and profitability as a cash crop (Prioty & Miah, 2024).

Despite its economic and agricultural importance, mung bean production is severely challenged by various diseases, with approximately 20 pathogens reported in Bangladesh. Among them, foot and root rot, caused by *Sclerotium rolfsii* and *Fusarium oxysporum*, is one of the most devastating soil-borne diseases affecting mung bean globally (Islam et al., 2021). *Sclerotium rolfsii* alone has been reported to cause up to 14.72% seedling mortality in mutant lines, significantly reducing plant stand and yield. Conventional disease management approaches, including chemical fungicides and resistant cultivars, have shown limited effectiveness due to environmental constraints and pathogen adaptability (Weller et al., 2002).

In recent years, biological control strategies have gained attention as sustainable alternatives to chemical treatments. *Trichoderma* spp., known for its antagonistic properties, has been widely used as a bio-fungicide to improve plant health and growth (Kubheka & Ziena, 2022). Additionally, organic amendments such as poultry manure enhance soil microbial diversity, promoting disease suppression and plant vigor (Babu & Deepika, 2023). Several studies have demonstrated the effectiveness of combining microbial antagonists with soil amendments in managing soil-borne diseases (Ramzan et al., 2016). However, research on integrated eco-friendly approaches for controlling foot and root rot in mung bean remains limited in Bangladesh.

This study aims to evaluate the combined efficacy of *Trichoderma*-based bio-fungicides and poultry manure in controlling foot and root rot disease in mung bean. By integrating biological control agents with organic amendments, this research seeks to provide a sustainable and effective alternative to chemical fungicides, contributing to improved disease management and enhanced crop productivity.

## Materials and methods

**Experimental Site and Duration**

The study was conducted at the Field Laboratory, Department of Plant Pathology, PSTU, where a naturally infested field with foot and root rot disease was selected for experimentation. The field trials and laboratory experiments were carried out during the growing season of January 2015.

**Experimental Design and Layout**

The experiment was arranged in a Randomized Complete Block Design (RCBD) with four replications. Each block was divided into five experimental units, with the following plot specifications: Plot size: 6 m² (3 m × 2 m); Spacing: Block to block: 1 m, Plot to plot: 50 cm, Row to row: 20 cm, Plant to plant: 5 cm; Treatments: Six organic amendments were evaluated- Poultry manure @ 0.5 kg/m², Cow dung @ 0.5 kg/m², Wood ash @ 0.5 kg/m², Vermicompost @ 1 kg/m², Mustard oil cake (MOC) @ 30 g/m², Sunflower oil cake (SOC) @ 50 g/m²

The amendments were incorporated 15–30 days before sowing to allow decomposition, microbial colonization, and disease suppression. A light irrigation was applied after amendment incorporation. Non-amended plots served as the control, and 400 BARI Mung-6 seeds were sown per plot.

**Isolation and Identification of the Pathogen**

Mung bean seedlings exhibiting foot and root rot symptoms were collected for pathogen isolation. The infected root samples were placed in sterilized poly bags and transported to the laboratory for further analysis.

The fungal pathogen was isolated following Carter et al. (1999). Infected root samples were washed under running tap water, surface sterilized with 0.1% sodium hypochlorite (NaOCl) for one minute, and rinsed three times with sterile distilled water. After drying on sterile filter paper, the roots were cut into 1 cm segments and placed on Potato Dextrose Agar (PDA) in Petri plates under aseptic conditions. The plates were incubated at room temperature for 6–8 days. Fungal colonies emerging from infected tissue were transferred to fresh PDA plates and incubated for an additional 7 days.

Based on cultural and morphological characteristics, the causal organism was confirmed as *Sclerotium rolfsii*, with no evidence of *Fusarium oxysporum*. The selected field was used for further experimentation.

In Vitro Evaluation of Trichoderma-Based Formulations Against *Sclerotium rolfsii*

The antagonistic potential of two formulated Trichoderma species was assessed: BAU-Biofungicide (*Trichoderma harzianum*); Commercially available *Trichoderma* suspension.

The dual culture method (Dennis & Webster, 1971) was used. PDA plates were inoculated with 2 mm mycelial plugs of *Sclerotium rolfsii* and *Trichoderma spp.*, placed at opposite edges of the plates. Plates were incubated at room temperature for seven days, and growth inhibition was recorded. Control plates contained only *Sclerotium rolfsii*.

*Calculation of Growth Inhibition*

The percentage inhibition of mycelial growth was calculated using Reddy et al. (1994):

$$\%\text{Inhibition} = \frac{X-Y}{X} \times 100$$

Where:

X = Control (untreated) growth measurement

Y = Treated growth measurement

Each treatment had four replications.

**Application of Bio-Agents Through Seed Treatment**

Mung bean seeds of BARI Mung-6 were obtained from the Bangladesh Agricultural Research Institute (BARI), Mymensingh. Seeds were slightly moistened to allow uniform adhesion of the biofungicide. BAU-Biofungicide was applied at **2**.5% of seed weight, ensuring complete surface coverage before sowing.

Observations and Data Collection

Seedling emergence was recorded at 7, 14, and 21 days after sowing (DAS). Disease assessments were conducted at 10, 20, and 30 DAS.

*Germination and Disease Incidence Calculations*

1. Emergence Percentage

$$\%\,Emergnece = \frac{Number\ of\ seedlings\ emerged\ per\ plot}{Total\ number\ of\ seeds\ sown\ per\ plot} x100$$

2. Pre-Emergence Seed Rot (PESR) Percentage

$$\%\,PESR = \frac{No.of\ seeds\ sown\ perplot - No.of\ seedlings\ emerged\ at\ 21\ DAS}{Number\ of\ seeds\ sown\ per\ plot} \text{x}100$$

3. Post-Emergence Seedling Mortality (POESM) Percentage (Kataria & Grover, 1976):

$$\%\,POESM = \frac{Number\ of\ affected\ seedlings\ per\ plot}{Total\ number\ of\ seedlings\ per\ plot} x100$$

At harvest, five randomly selected plants per plot were uprooted to measure: Plant height (cm), Pods per plant, Seeds per pod, 1000-seed weight (g), Seed yield (kg/ha)

**Data Analysis**
The collected data were analyzed using Analysis of Variance (ANOVA) to determine treatment effects. Least Significant Difference (LSD) Test was used at a 5% significance level (Anderson et., 2001) to compare means. Statistical analyses were performed using MSTAT-C software.

## Results

*Evaluation of Bio-Agents (In Vitro)*
The inhibitory effects of two *Trichoderma*-based formulations on the radial mycelial growth of *Sclerotium rolfsii* were assessed in vitro (Table 1). The results demonstrated that *Trichoderma* suspension and BAU-Biofungicide significantly reduced mycelial growth to 30.75 mm and 26.00 mm, respectively, compared to 90.0 mm in the control, where the pathogen exhibited unrestricted growth.The difference in mycelial growth reduction between the two *Trichoderma* treatments was statistically non-significant (marked with the same letter "b"), indicating their similar efficacy. BAU-Biofungicide exhibited the highest mycelial growth inhibition (71.11%), followed by *Trichoderma* suspension (65.83%).
This suppression is likely attributed to antifungal metabolites such as antibiotics and volatile organic compounds that diffuse through the medium and hinder pathogen growth.

**TABLE 1. Efficacy of two formulations of *Trichoderma* on growth of *Sclerotium rolfsii***

| Treatment | Radial mycelial growth (mm) | Inhibition of mycelial growth (%) |
|---|---|---|
| *Trichoderma* suspension | 30.75 b | 65.83 |
| BAU- Biofungicide | 26.00 b | 71.11 |
| Control | 90.0 a | 00 |
| LSD at P0.05 | 19.0 | |

Treatment means are average of four replications. In a column figures with similar letter do not differ significantly

Evaluation of Organic Amendments and BAU-Biofungicide Under Field Conditions

**Effect of Organic Amendments on Field Emergence of Mung Bean**
The effect of organic amendments on mung bean emergence was recorded at 7, 14, and 21 days after sowing (DAS) (Table 2). Results showed that poultry litter and vermicompost significantly improved field emergence compared to the control At 7 DAS, poultry litter had the highest emergence rate (41.81%), followed by vermicompost (39.19%), while the control had the lowest (26.06%). By 21 DAS, poultry litter (75.0%) and vermicompost (70.0%) continued to show the highest emergence rates, while the control remained the lowest (60.0%). The statistical analysis (LSD at P<0.05) confirmed significant differences among treatments, with poultry litter and vermicompost being the most effective amendments.

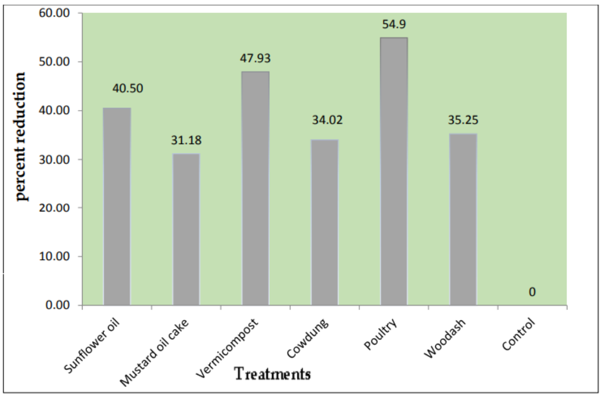


Fig 1. Effect of soil amendment on percent reduction of total mortality over control

**Effect of Organic Amendments on Pre- and Post-Emergence Mortality of Mung Bean**
The impact of organic amendments on pre-emergence seed rot (PESR) and post-emergence seedling mortality (POESM) was evaluated (Table 3). Results showed pre-emergence mortality was lowest in poultry litter (25.0%), followed by vermicompost (30.0%), while the control recorded the highest (40.0%). Post-emergence mortality at 10 DAS was significantly lower in poultry litter (7.5%) and vermicompost (8.0%) compared to the control (20.0%). By 30 DAS, seedling mortality remained lowest in poultry litter (3.00%) and vermicompost (3.50%), while the control had the highest (11.0%). Poultry litter resulted in the highest total mortality reduction (54.9%), followed by vermicompost (47.93%) (Fig. 1).

**Effect of Organic Amendments on Yield and Yield-Related Parameters of Mung Bean**
The application of organic amendments significantly influenced plant height, number of pods per plant, number of seeds per plant, 1000-seed weight, and grain yield (Table 4). Results showed poultry litter produced the tallest plants (33.00 cm), highest pod count (12.80), and highest seed count per plant (96.75). Vermicompost and sunflower oil cake also improved plant growth and

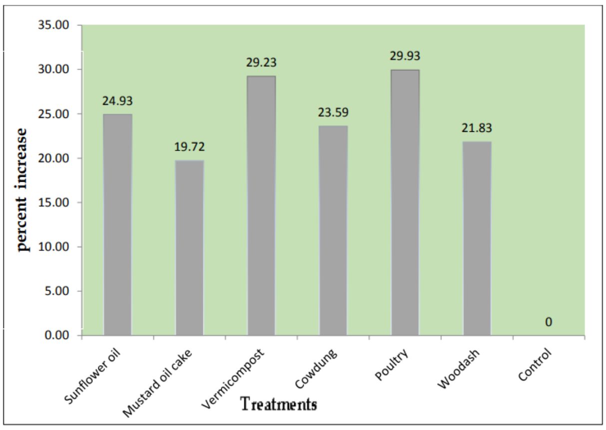


Fig. 2. Effect of soil amendment on percent increase of yield over control

**Table 2. Effect of organic amendment of soil on field emergence of mungbean**

| Treatments | Emergence (%) | | |
|---|---|---|---|
| | 7 DAS | 14 DAS | 21 DAS |
| Sunflower oil-cake | 36.50 ab | 64.69 bc | 66.0 c |
| Mustard oil-cake | 34.00 b | 59.25 d | 62.75 d |
| Vermicompost | 39.19 ab | 68.31 ab | 70.0 b |
| Cowdung | 37.75 ab | 62.25 cd | 65.0 c |
| Poultry litter | 41.81 a | 69.00 a | 75.0 a |
| Woodash | 36.25 ab | 62.31 cd | 63.25 d |
| Control | 26.06 c | 50.56 e | 60.0 e |
| LSD at P0.05 | 5.97 | 3.83 | 1.56 |
| CV% | 11.19 | 4.13 | 1.60 |

Treatment means are average of four replications. In a column figures with similar letter do not differ significantly

**Table 3. Effect of organic soil amendment on seedling mortality of mungbean**

| Treatment | Seed rot/Seedling Mortality (%) | | | | Total mortality (%) |
|---|---|---|---|---|---|
| | PESR (%) | POESM (%) | | | |
| | 21 DAS | 10 DAS | 20 DAS | 30 DAS | |
| Sunflower oil cake | 34.0 d | 8.50 c | 10.25 c | 5.0 bc | 57.75 |
| Mustard oil cake | 37.00 b | 10.25 b | 12.75 b | 6.75 b | 66.75 |
| Vermicompost | 30.0 e | 8.00 c | 9.0 c | 3.50 cd | 50.5 |
| Cowdung | 35.0 c | 10.00 b | 12.75 b | 6.25 b | 64.0 |
| Poultry litter | 25.0 f | 7.50 c | 8.25 c | 3.00 d | 43.75 |
| Woodash | 35.25 c | 11.0 b | 12.25 b | 6.25 b | 64.75 |
| Control | 40.0 a | 20.0 a | 26.0 a | 11.0 a | 97 |
| LSD P0.05 | 0.73 | 1.49 | 1.93 | 1.84 | |
| CV% | 1.46 | 9.30 | 9.99 | 10.80 | |

Figures are means of four replications. Figures in parenthesis are the percent reduction of pre-emergence mortality over control. PESR= Pre-emergence seed rot; PoESM= Post-emergence seedling mortality. In a column figures with similar letter do not differ significantly at 0.05% by LSD.

**Table 4. Effect of soil amendment on yield and yield related parameters of mungbean.**

| Treatment | Plant height (cm) | No. of pod /plant | No. of seeds /plant | Thousand seeds wt. (gm) | Yield (kg/ha) |
|---|---|---|---|---|---|
| Sunflower oil cake | 30.50 b | 11.95 ab | 89.30 b | 57.25 ab | 887.5 b |
| Mustard oil cake | 24.88 d | 9.45 cd | 79.80 cd | 47.25 cd | 850.0 d |
| Vermicopost | 30.50 b | 11.65 ab | 90.75 b | 59.0 a | 917.5 a |
| Cowdung | 27.80 c | 9.15 cd | 86.30 b | 52.50 bc | 877.5 bc |
| Poultry litter | 33.00 a | 12.80 a | 96.75 a | 60.50 a | 922.5 a |
| Wood-ash | 27.20 c | 10.00 c | 84.03 bc | 50.50 cd | 865.0 cd |
| Control | 24.75 d | 8.05 d | 76.40 d | 45.0 d | 710.0 e |
| LSD P0.05 | 1.62 | 1.66 | 5.97 | 6.050 | 19.0 |
| CV% | 3.84 | 10.90 | 4.69 | 7.66 | 1.48 |

Treatment means are average of four replications. In a column figures with similar letter do not differ significantly at P 0.05% by LSD yield-related parameters significantly. The highest grain yield was recorded in poultry litter-treated plots (922.5 kg/ha), followed by vermicompost (917.5 kg/ha). Yield increases over control ranged from 19.72% to 29.93%, with poultry litter showing the highest increase (29.93%) (Fig. 2).

## Discussion

### Antagonistic Potential of Trichoderma-Based Biofungicides

The findings confirm the strong antagonistic activity of *Trichoderma* spp. against *Sclerotium rolfsii*. The significant reduction in mycelial growth observed in vitro aligns with previous studies highlighting the biocontrol potential of *Trichoderma* through mechanisms such as: Antibiosis (production of antifungal metabolites), Mycoparasitism (direct attack on pathogens), Competitive exclusion in the rhizosphere, and induction of plant defense responses (Kubheka & Ziena, 2022). These results support previous findings on the effectiveness of *Trichoderma harzianum* against fungal pathogens in pulses and other crops (Hannan et al., 2012).

### Impact of Organic Amendments on Field Emergence and Seedling Survival

The significant improvement in field emergence and reduced mortality in poultry litter- and vermicompost-amended plots can be attributed to enhanced soil microbial activity, nutrient availability, and pathogen suppression. These findings align with previous studies demonstrating the role of organic amendments in improving seed germination and early seedling establishment (Adnani et al., 2024).

The reduced pre- and post-emergence mortality in organic amendment-treated plots suggests that poultry litter and vermicompost help suppress *S. rolfsii* and improve soil health, a trend also reported in studies on legumes and vegetable crops (Faruk et al., 2020).

### Effects on Yield and Yield-Related Parameters

The substantial increases in plant height, pod number, and yield in poultry litter- and vermicompost-treated plots support previous research highlighting the benefits of organic soil amendments for improved crop productivity (Asghar & Kataoka, 2021; Tahir et al., 2024).

The increased yield observed in poultry litter (29.93% over control) suggests that nutrient-rich organic amendments enhance plant growth and disease resistance. The findings are consistent with studies demonstrating that Trichoderma-treated seeds, when grown in soil amended with organic matter, exhibit superior growth and productivity in various crops (Uddin et al., 2011).

### Future Research Directions

While the study confirms the efficacy of *Trichoderma*-based biofungicides and organic amendments, future research should focus on field trials under different agroecological conditions to validate results, optimizing application rates and methods for maximum disease suppression, and assessing long-term effects on soil fertility and microbial biodiversity.

## Conclusion

The integration of *Trichoderma*-based biofungicides with organic amendments such as poultry litter and vermicompost offers a sustainable strategy for managing *Sclerotium rolfsii* and improving mung bean productivity. Future research should explore the scalability and cost-effectiveness of these practices to promote their widespread adoption in sustainable agriculture.

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