**Antagonistic Potential of Biocontrol Agents against Macrophomina phaseolina in Green Gram**

**ABSTRACT**

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| Macrophomina blight caused by Macrophomina phaseolina, is one of the most destructive diseases affecting green gram (Vigna radiata L.) particularly under drought and high-temperature conditions. The ability of the pathogen to persist in the soil and rapidly colonize host tissues makes management difficult through conventional means. In an effort to identify effective biological alternatives an *in vitro* study was conducted to evaluate the antagonistic performance of selected fungal and bacterial bioagents against M. phaseolina. The experiment involved six biocontrol agents four fungal species from the Trichoderma genus and two bacterial strains, Bacillus subtilis and Pseudomonas fluorescens. Dual culture and streak plate techniques were employed on PDA medium to assess their efficacy. Results revealed significant variation in pathogen suppression, with Trichoderma harzianum exhibiting the highest growth inhibition at 87.77%, followed by Trichoderma hamatum and Trichoderma asperellum. In contrast, the bacterial antagonists showed comparatively lower inhibition levels. The outcomes of this study highlight the potential of Trichoderma harzianum as a promising biocontrol agent for managing Macrophominablight in green gram under eco-friendly disease management practices. |

***Key words:*** *Vigna radiata, Macrophomina phaseolina, Biocontrol agents, Trichoderma*

1. **INTRODUCTION**

Pulses, a remarkable gift from nature, plays a dual role in sustaining soil health and fertility while serving as a highly nutritious food source. Their benefits make them an indispensable element of sustainable agriculture in arid tropical regions. Pulses belong to the Fabaceae family and their mature dry seeds are vital component of the human diet. Renowned for their high protein content, pulses also enhance soil fertility by fixing atmospheric nitrogen through symbiotic associations with nitrogen-fixing *Rhizobacteria*. (Muchomba *et al*. 2023).

Green gram also called as Mung bean is a versatile and widely cultivated crop grown across various regions in India. "In 2023–2024, green gram occupied 5.50 million hectares area in India, with Maharashtra accounting for 8–9% of the total cultivated area. Production of green gram in India in 2023-2024 is 3.03 **million metric tons** MMT and for Maharashtra 0.24 MMT. India's *kharif* productivity for green gram in year 2023-2024 is 600-700 kg/ha. (Ministry of Agriculture and Farmers Welfare. (2024). Final estimates of production of major agricultural crops for 2023-24. Government of India.) Green gram is a highly nutritious pulse, rich in proteins, carbohydrates, dietary fiber, essential vitamins and minerals, while being naturally low in fat. Due to its high protein content, it serves as an excellent plant-based alternative to meat, particularly for vegetarian diets. Beyond its nutritional value, green gram is known for a range of health-promoting properties, including antioxidant, anti-inflammatory, anticancer and cholesterol-lowering effects. (Mekkara and Bukkan 2021)

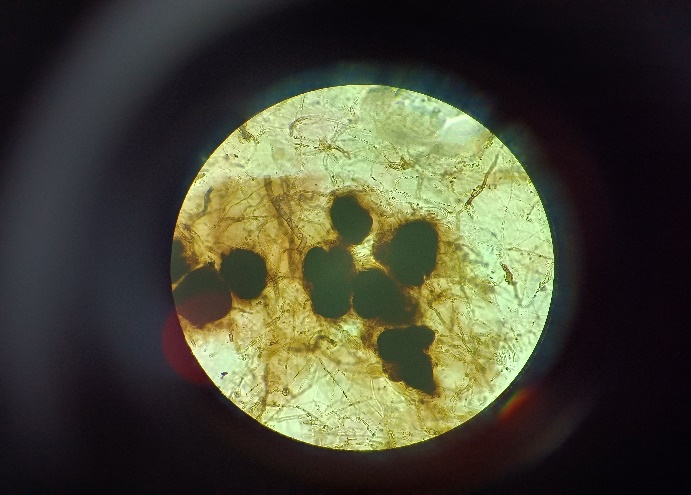
Mung bean is a rich source of essential minerals like potassium and phosphorus and provides high-quality protein, making it a valuable crop for nutritional security. India ranks among the top global producers of mung bean (Mallaiah and Krishna, 2018).

Globally, India is the major producer as well as user of green grams. Its output makes up between 10-12 percent overall pulse production of the nation. "Mung bean productivity is constrained by foliar fungal pathogens, demanding effective biocontrol solutions." For most people pulses constitute an affordable and high-quality source of protein. "India alone accounts for 22% of global pulse production and 33% of the world’s pulse cultivation area." (Sandhu and Dhaliwal. 2016)

In Maharashtra, fungal crop diseases such as *Macrophomina* blight and powdery mildew are considered among the most damaging, often leading to substantial financial losses for farmers (Zote *et al*., 1983).

Macrophomina phaseolina is considered a heat-favoring pathogen, with its disease impact becoming more severe when soil temperatures increase between 28°C and 35°C, especially when soil moisture is low (Gary *et al*., 1991).Macrophomina phaseolina-induced leaf blight in green gram has shown to significantly impair plant development, causing reductions of 9.38% in height, 26.32% in leaf count, 30% in pod formation and 40% drop in pod weight per plant (Tandel *et al*., 2010).

Eco-friendly and non-chemical approaches are increasingly gaining importance as sustainable alternatives to conventional chemical-based plant disease management. While fungicides have been used to manage Macrophomina blight in green gram, their repeated application poses risks such as environmental degradation and the development of fungicide-resistant pathogen strains. Given the economic importance of green gram in India and the significant yield losses attributed to Macrophomina phaseolina, the present investigation was undertaken to assess the *in* *vitro* efficacy of various biocontrol agents against the causal pathogen.

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**Fig 1B. Microsclerotia of M. phaseolina**

**Fig. 1 A. Pure culture of *M. phaseolina***

1. **MATERIAL AND METHODS**

Blight-infected samples collected from Dr. Sharadchandra Pawar college of agriculture baramati research field were washed with tap water and placed on blotting paper to dry. In a laminar air flow chamber, sterilized PDA medium was poured into Petri plates and left to solidify. Small pieces of infected tissue, containing both healthy and diseased parts, were cut with a sterile blade and surface sterilized using sodium hypochlorite for two minutes. After rinsing three times with sterile distilled water, the pieces were blot-dried and aseptically placed on PDA plates.

The plates were incubated at 27 ± 2°C and fungal growth was observed after 6–8 days. The pathogen was purified using the single hyphal-tip method and sub-cultured on fresh PDA. Identification was done based on colony characteristics and microscopic features, especially the shape, size and the colour of sclerotia (Chavan *et al*. 2023).

**Pathogenicity test**

Pathogenicity of *Macrophomina phaseolina* were proved *in-vitro* (pot culture) by applying Koch's postulates. Surface sterilized seeds of *Macrophomina phaseolina* susceptible mungbean Cv. JL-781 were sown 10 seed per pot in the earthen pots (30 cm diameter) filled with steam sterilized potting mixture of soil: sand: Farm Yard Manure (2:1:1). After a week healthy growing mungbean seedlings in pot were maintained, by watering regularly and keeping in the green house for further growth. Spore-cum-mycelial suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was suitably diluted with sterile distilled water to get an inoculum concentration of 5 x 106 spores/ml. Thirty days old seedling of mungbean Cv. JL-781 were artificially inoculated by spraying the sclerotial suspension (5 x 106 sclerotia/ml) of the test pathogen using an atomizer. Seedlings sprayed with sterile water (without inoculum) were also maintained as suitable control experiment. Inoculated pot were placed in the green house and polythene bag is wrapping around the inoculated pot where high humidity (>80%) and optimum temperature (27 ± 2 °C) were maintained for further development of blight symptoms. Pathogen was reisolated from the characteristic blight symptoms developed on artificially inoculated mungbean leaves. The resulting cultures were compared with the original inoculants to fulfill Koch’s postulates.

**Antimicrobial efficacy of bioagents**

The efficacy of six biocontrol agents Trichoderma harzianum, Trichoderma asperellum, Trichoderma hamatum, Trichoderma virens, Pseudomonas fluorescens and Bacillus subtilis against M. phaseolina was evaluated through the dual culture technique.

**Dual Culture Technique**

Potato Dextrose Agar (PDA) was prepared and sterilized at 121°C and 15 psi for 20 minutes. About 20 ml of the medium was poured into 90 mm sterile Petri plates. Once solidified 5 mm disc from the periphery of the growing culture of *Macrophomina phaseolina* was placed near the edge of the plate. On the opposite side, a disc of the fungal biocontrol agent was placed at an equal distance. For bacterial antagonists, the culture was streaked on the side opposite the pathogen. Plates with only *M. phaseolina* served as controls.

**Experimental Procedure**

All inoculated Petri dishes were incubated at a temperature of 27 ± 2°C for a period of seven days. Each treatment was carried out in triplicate. Observations were recorded once the fungal growth in the untreated control plates reached the full diameter of 90 mm. The radial growth of M. phaseolina was measured in each treatment and the percentage inhibition of fungal growth compared to the control was calculated using the formula proposed by (Vincent 1947).

Where, I= Per cent Inhibition of fungal growth

C= Colony diameter (mm) in control plate

T= Colony diameter (mm) in treated plate

1. **RESULTS AND DISCUSSION**

The mycoparasitic ability of six biocontrol agents, including four fungal species (*Trichoderma* spp.) and two bacterial species (*Bacillus subtilis* and *Pseudomonas fluorescens*) was examined for their antagonistic activity against *Macrophomina phaseolina*. The evaluation was carried out by employing dual culture on the PDA Media.The results showed considerable differences in the efficacy of the biocontrol agents tested. Among six bioagents tested, *Trichoderma harzianum* demonstrated the highest inhibition of growth of pathogen with a colony diameter of 11.00 mm and a growth inhibition percentage of 87.77 per cent which is best compared to the other treatments. This was followed by *Trichoderma hamatum,* which exhibited a colony diameter of 49.00 mm and a growth inhibition of 45.55 per cent.

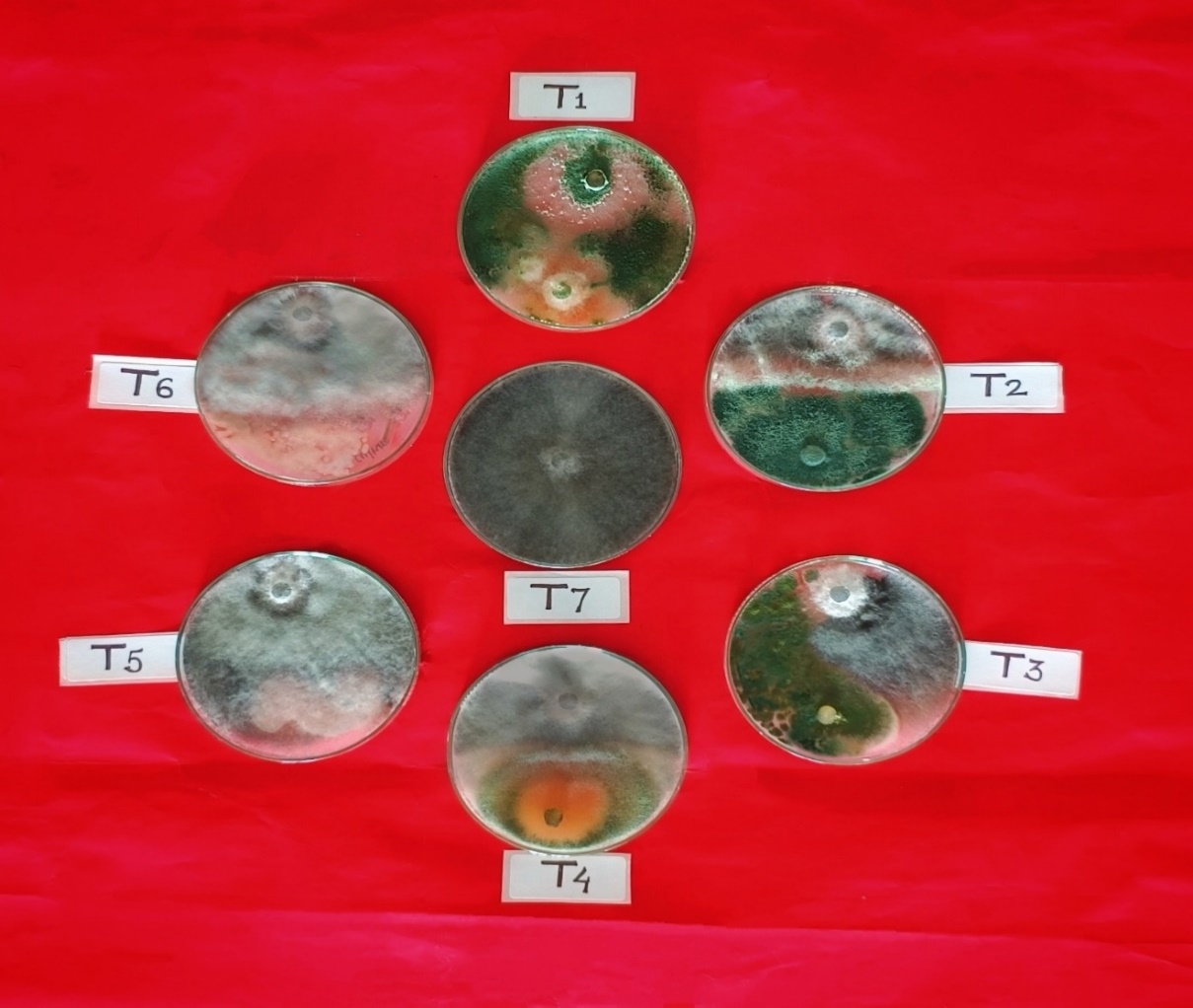
*Trichoderma asperellum* and bacterial bioagents *Bacillus subtilis* showed moderate efficacy with colony diameter of 60.50 mm and 65.83 mm and growth inhibition rates of 32.77 per cent and 27.41 per cent, respectively and *Trichoderma virens* showed colony diameter 74.00 mm and growth inhibition 17.77 per cent. The bacterial bioagent *Pseudomonas fluorescens* were the least effective with colony diameter of 78.33 mm and growth inhibition rates of 12.96 per cent.

These findings support the observations made by several earlier researchers. Singh *et al.* (2007) examined the inhibitory effects of *Trichoderma* species under laboratory conditions against *M. phaseolina* and found that *Trichoderma harzianum* exhibited the highest inhibition rate 71.85 per cent and reported that *Trichoderma harzianum* showed the best performance.

**Table 1. Efficacy of various bioagents against *Macrophomina phaseolina* under *in vitro* conditions*.***

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| **Treatment number** | **Treatments** | **Colony Diameter of the Pathogen\* (mm)** | **% Growth Inhibition** |
| T1 | *Trichoderma harzianum* | 11.00 | 87.77 |
| T2 | *Trichoderma asperellum* | 60.50 | 32.77 |
| T3 | *Trichoderma hamatum* | 49.00 | 45.55 |
| T4 | *Trichoderma virens* | 74.00 | 17.77 |
| T5 | *Pseudomonas fluorescens* | 78.33 | 12.96 |
| T6 | *Bacillus subtilis* | 65.83 | 27.41 |
| T7 | Control | 90.00 | - |
|  | **S.E.(m)±** | **0.51** | - |
|  | **C.D. (0.05)** | **1.57** | - |

\*Mean of three replication.



**Plate 1. *In vitro* efficacy of various bioagents against *M. phaseolina***

**Fig.2 *In vitro* evaluation of biocontrol agents against *Macrophomina phaseolina***

**Pathogencity Test**



**Fig 3. A. Uninoculated healthy B. infected plant after inoculation**

**Fig 3C. Infected region**

**4. CONCLUSIONS**

The *in vitro* assessment of six biocontrol agents indicated that Trichoderma harzianum strong antagonistic activity and achieved the highest inhibition of mycelial growth against the target pathogen. Bacterial biocontrol agents are least effective against *M. phaseolina* under *in vitro* condition and pathogenicity is proved by spore suspension method.

**DISCLAIMER**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts. COMPETING INTERESTS: Authors have declared that no competing interests exist.

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