



***In-vitro* evaluation of natural farming inputs against *Macrophomina phaseolina* causing dry root rot of Green gram**

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Abstract

Green gram (*Vigna radiata* L.) is an important short-duration pulse crop cultivated widely in India, but its productivity is affected by several fungal diseases, including dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. The present study was undertaken to evaluate the *in-vitro* antifungal activity of natural farming inputs against *M. phaseolina*. The laboratory experiment was conducted in the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. Seven natural farming inputs, viz., Ghan Jeevamrit, Jeevamrit, Neem cake, Beejamrit, Cow urine, fermented buttermilk and Ramban, were evaluated at 20 per cent concentration using the poisoned food technique. The experiment was conducted in a Completely Randomized Design with three replications, and radial mycelial growth was recorded on the seventh day after incubation. Considerable variation was observed among the treatments in their ability to suppress the mycelial growth of *M. phaseolina*. Beejamrit recorded the minimum colony diameter of 9.87 mm and the maximum mycelial growth inhibition of 89.04 per cent, followed by Jeevamrit, which recorded 24.17 mm colony diameter and 73.15 per cent inhibition. Cow urine recorded 46.27 mm colony diameter with 48.59 per cent inhibition, followed by Neem cake with 58.67 mm growth and 34.81 per cent inhibition. Ghan Jeevamrit and Ramban recorded 62.33 and 68.77 mm colony diameter, corresponding to 30.74 and 23.59 per cent inhibition, respectively. Fermented buttermilk showed the lowest inhibitory effect among the natural farming inputs, with 75.33 mm colony diameter and 16.30 per cent inhibition, whereas the untreated control recorded 90.00 mm radial growth. The findings demonstrated considerable differences in the antifungal activity of the tested natural farming inputs, with Beejamrit and Jeevamrit showing greater suppression of *M. phaseolina* under *in-vitro* conditions.

Keywords: Green gram, *Macrophomina phaseolina*, dry root rot, natural farming inputs, beejamrit, jeevamrit

Introduction

Green gram (*Vigna radiata* L.), commonly known as mung bean, is an important short-duration pulse crop widely cultivated in India and other parts of Asia. Its adaptability to different agro-climatic conditions and cultivation during Kharif, Rabi and summer seasons make it an important component of pulse-based cropping systems. Besides providing dietary protein and other nutrients, mung bean contributes to soil fertility through biological nitrogen fixation and therefore has considerable importance in sustainable agriculture.

Despite its importance, mung bean productivity is constrained by several biotic stresses, particularly fungal diseases. Among these, dry root rot or charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is an important disease of mung bean, particularly under hot and moisture-deficient conditions. Iqbal and Mukhtar (2014) [4] reported that charcoal rot caused by *M. phaseolina* is an important disease of mung bean, especially in arid regions, and also demonstrated considerable pathogenic variability among isolates collected from mung bean.

Macrophomina phaseolina is a soil- and seed-associated pathogen with a broad host range. The fungus survives mainly as resistant black microsclerotia in soil and infected plant debris, enabling it to persist under unfavourable conditions. High temperature and moisture stress favour disease development. Following infection, the pathogen

colonizes root and basal stem tissues, resulting in impaired water and nutrient movement and progressive plant drying. The formation of microsclerotia within infected tissues is a characteristic feature of the disease (Gupta *et al.* 2012; Iqbal & Mukhtar, 2014) [3, 4].

In mungbean, infected plants may exhibit yellowing and wilting followed by drying of foliage, root discoloration and decay, and development of black microsclerotia on infected tissues. Severely affected plants become stunted, dry prematurely and can be easily uprooted because of extensive root deterioration. Disease incidence may become particularly severe when high temperature coincides with inadequate soil moisture. Anupriya *et al.* (2024) [1] reported dry root rot incidence of approximately 30–40% in mungbean and evaluated organic amendments, plant extracts and bio-agents as alternatives for disease management.

Management of dry root rot is difficult because the pathogen survives in soil and crop residues for extended periods and possesses considerable pathogenic variability. Although chemical fungicides can provide disease suppression, their repeated and indiscriminate use may adversely affect soil microorganisms, environmental quality and long-term disease-management sustainability. Consequently, greater emphasis is being placed on biological, organic and natural farming-based approaches for managing soil-borne diseases.

Therefore, the present study was undertaken to evaluate Neem cake, Jeevamrit, Beejamrit, Ghanjeevamrit, Cow urine, Ramban and Sour buttermilk *in vitro* against *M. phaseolina*, with the aim of identifying promising natural farming inputs for the eco-friendly management of dry root rot of mungbean.

Materials and Methods

Experimental site

The laboratory experiments were carried out in the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

Natural Farming Inputs used Against *Macrophomina phaseolina*

1. Beejamrit
2. Jeevamrit
3. Ghan Jeevamrit
4. Neem cake
5. Fermented buttermilk
6. Cow urine
7. Ramban

Preparation of Natural Farming Inputs

The natural farming inputs used in the present study were prepared at Natural and Organic Farming Research and Training Centre, VNMKV, Parbhani.

Beejamrit was prepared by suspending 5 kg fresh indigenous cow dung in 20 L water for 12 h, followed by addition of lime solution (50 g L⁻¹), 5 L cow urine and a handful of field soil, and was used for seed treatment (Palekar, 2006) [6]. Jeevamrit was prepared by mixing 10 kg cow dung, 10 L cow urine, 2 kg jaggery, 2 kg pulse flour and 1 kg fertile soil in 200 L water, followed by seven days of shaded fermentation with regular stirring; the filtrate was used for foliar application (Somdutt, 2021) [11]. Ghanjeevamrit was prepared from 10 kg cow dung, 1–2 kg jaggery, 1–2 kg gram flour and fertile soil, with cow urine, followed by 72–96 h fermentation and shade drying (Sharma & Chadak, 2022) [10]. Neem cake was obtained as the dried solid residue after oil extraction from neem (*Azadirachta indica*) seeds. Fermented buttermilk was prepared by churning curd with water and fermenting it under shade for 3–5 days, followed by filtration. Ramban was prepared by mixing 8 L Jeevamrit and 3 L fermented buttermilk and making the volume up to 100 L with water; it was filtered and used within 48 h as a foliar spray (National

Institution for Transforming India, 2026). Fresh cow urine was collected directly into clean, sterile bottles from cows maintained at the Natural and Organic Farming Research & Training Centre, VNMKV, Parbhani, and stored until use.

Disease sample

Green gram plants showing typical symptoms of dry root rot were collected from farmers field and aseptically inoculated on Potato Dextrose Agar medium for tissue isolation.

In-vitro evaluation of natural farming inputs against *M. phaseolina* (Poisoned food technique)

Seven natural farming inputs, viz., Ghan Jeevamrit, Jeevamrit, neem cake, Beejamrit, cow urine, fermented milk and Ramban, were evaluated at 20% concentration against *Macrophomina phaseolina* using the poisoned food technique (Nene & Thapliyal, 1979) [5] under aseptic conditions. Each preparation was filtered through double-layered muslin cloth, and the filtrate was considered as the stock solution. The stock solutions were autoclaved at 121°C and 15 psi for 20 min. The required quantity was incorporated into molten Potato Dextrose Agar (PDA) to obtain a 20% concentration and poured into sterilized Petri plates. A 7-mm mycelial disc from a 7-day-old actively growing culture of *M. phaseolina* was placed at the centre of each plate. Plates were incubated at 25 ± 2°C, and radial mycelial growth was recorded on the 7th day. The experiment was conducted in a Completely Randomized Design (CRD) with three replications. Percentage inhibition of mycelial growth was calculated using the formula of Vincent (1947) [14]:

$$\text{PGI (\%)} = [(C - T) / C] \times 100$$

Where, PGI = percentage growth inhibition, C = radial growth in control, and

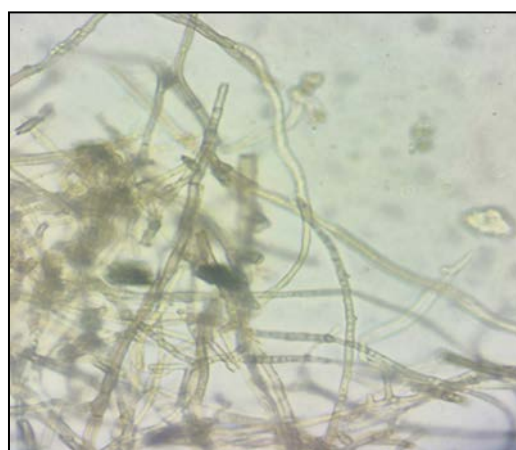
T = radial growth in treatment

Treatment Details:

Treatment No	Natural Farming Inputs	Concentration
T ₁	Ghan jeevamrit	@20% conc.
T ₂	Neem cake	@ 20% conc
T ₃	Jeevamrit	@ 20% conc
T ₄	Beejamrit	@ 20% conc
T ₅	Cow urine	@ 20% conc
T ₆	Fermented buttermilk	@ 20% conc
T ₇	Ramban	@ 20% conc
T ₈	Control	@ 20% conc



(A)



(B)



(C)

Plate 1: *Macrophomina phaseolina* (A) Pure culture (B) Septate hyphae (C) Natural Farming Inputs

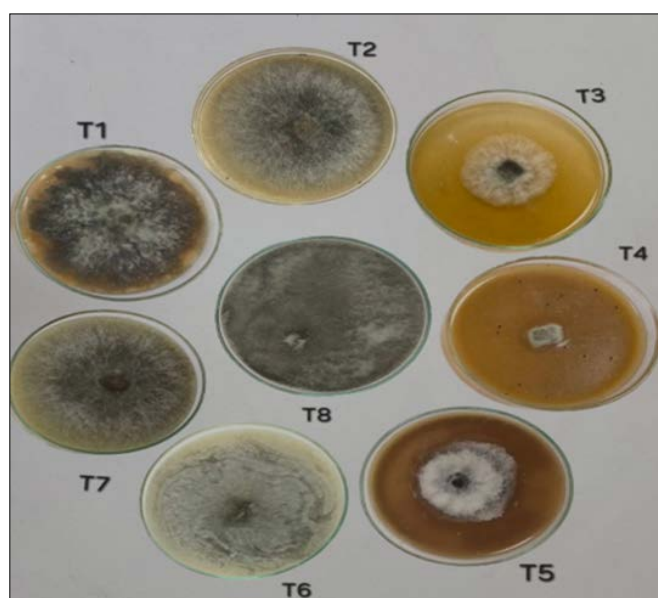


Plate 2: *In-vitro* Evaluation of Natural Farming Inputs against *Macrophomina phaseolina* using the Poisoned Food Technique

Results

In vitro efficacy of natural farming inputs against *Macrophomina phaseolina* by poison food technique.

Efficacy of seven different Natural farming inputs viz., Ghan Jeevamrit, Jeevamrit, neem cake, Beejamrit, cow urine, Fermented buttermilk, ramban will be evaluated @ 20% concentration were tested against *Macrophomina phaseolina in-vitro*. Results obtained were tabulated in Table 1. Illustrated in fig 1 and Plate.2.

Mycelial growth

The data presented in Table 1. revealed considerable variation among the treatments with respect to mycelial growth of the pathogen. The minimum mycelial growth was recorded in T₄ (Beejamrit @ 20%), with a colony diameter of 9.87 mm, followed by T₃ (Jeevamrit @ 20%) with 24.17 mm colony diameter. The treatments T₅ (cow urine @ 20%) and T₂ (Neem cake @ 20%) recorded mycelial growth of 46.27 and 58.67 mm, respectively. Further, T₁ (Ghan jeevamrut @ 20%) and T₇ (Ramban @ 20%) recorded

colony diameters of 62.33 and 68.77 mm, respectively. The maximum mycelial growth among the treated plates was observed in T₆ (Fermented buttermilk @ 20%), with a colony diameter of 75.33 mm. In the untreated control T₈, the pathogen showed complete radial growth of 90.00 mm.

Mycelial growth Inhibition

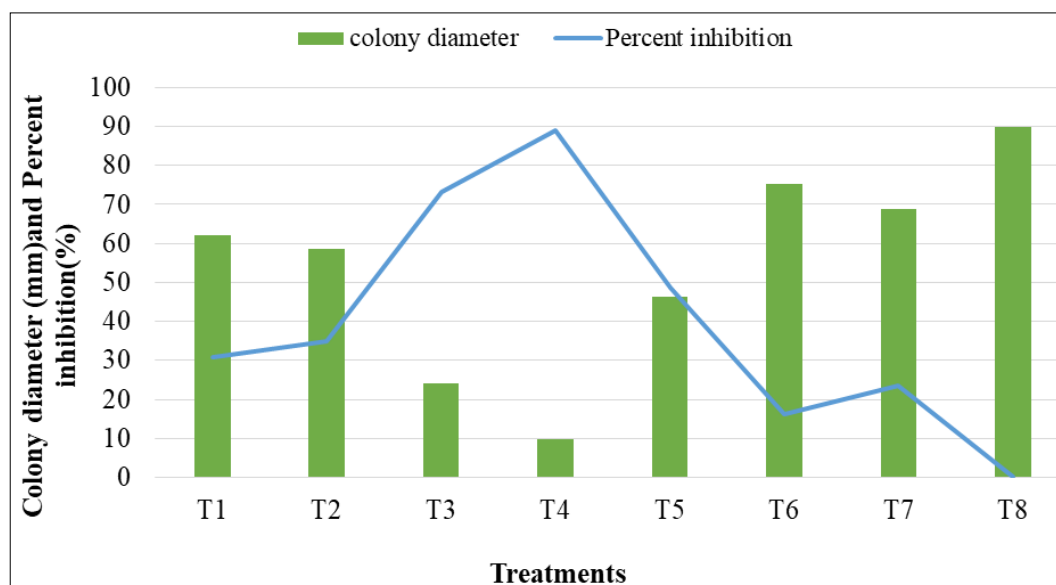
The highest mycelial growth inhibition was recorded in T₄ (Beejamrit @ 20%), which showed 89.04 per cent inhibition, followed by T₃ (Jeevamrit @ 20%) with 73.15 per cent inhibition. T₅ (cow urine @ 20%) recorded 48.59 per cent inhibition, whereas T₂ (Neem cake @ 20%) showed 34.81 per cent inhibition. The inhibition recorded by T₁ (Ghan jeevamrut @ 20%) and T₇ (Ramban @ 20%) was 30.74 and 23.59 per cent, respectively. The lowest inhibition among the natural farming inputs was observed in T₆ (Fermented buttermilk @ 20%), with 16.30 per cent inhibition. As expected, the untreated control T₈ recorded 0.00 per cent inhibition.

Table 1: *In-vitro* efficacy of different Natural Farming Inputs against *Macrophomina phaseolina* on 7 DAI.

Tr. no.	Natural Farming Inputs	Conc (%)	Colony Diameter(mm)	Percent Inhibition
T ₁	Ghan jeevamrit	20 %	62.33	30.74 (33.65)
T ₂	Neem Cake	20 %	58.67	34.81 (36.14)
T ₃	Jeevamrit	20 %	24.17	73.15 (58.76)
T ₄	Beejamrit	20 %	9.87	89.04 (70.69)
T ₅	cow urine	20 %	46.27	48.59 (44.17)
T ₆	Fermented Buttermilk	20 %	75.33	16.30 (23.78)
T ₇	Ramban	20 %	68.77	23.59 (29.03)
T ₈	Control	-	90.00	00.00 (00.00)
	SE ±		0.83	0.67
	CD @ 1 %		2.52	2.02

*Figures in parenthesis are arcsine transformed values. Mean of three replications.

*DAI: Days after Incubation

**Fig 1:** *In-vitro* efficacy of different Natural Farming Inputs against *Macrophomina phaseolina* on 7 DAI. (Poison food technique)

The present findings are consistent with the observations of Rajeswari *et al.* (2018) ^[8], who demonstrated the antifungal potential of natural farming inputs against *Rhizoctonia solani* under *in vitro* conditions. Their study showed that both Jeevamrutham and Beejamrutham completely inhibited mycelial growth at a 5% concentration. At lower concentrations, Jeevamrutham reduced fungal growth by 61.48% at 2% and 37.70% at 0.5%, whereas Beejamrutham recorded 56.29% inhibition at 3% and 36.29% at 1%.

The present findings are in agreement with Srinivas *et al.* (2021) ^[13], who reported that neem cake effectively inhibited the mycelial growth of *Macrophomina phaseolina*, recording 38.52, 58.34 and 62.96 per cent inhibition at 5, 10 and 20 per cent concentrations, respectively. Neem cake recorded the highest mean inhibition (53.27%), followed by castor cake (47.22%),

Prakash *et al.* (2017) ^[7] evaluated cow urine against *Sclerotium oryzae* under *in vitro* conditions. Cow urine showed strong antifungal activity, completely inhibiting mycelial growth at 5%, 7.5%, and 10% concentrations. Even at lower concentrations, it recorded high inhibition (98.14% at 2.5% and 63.7% at 1.25%), indicating its strong potential for suppressing the pathogen.

Similarly, Saharan *et al.* (2023) ^[9] reported the inhibitory effect of Ghan Jeevamrit against soil-borne fungal pathogens, while Joshi and Sharma (2018) and Upadhyay and Tiwari (2019) documented the antifungal activity of cow urine against several plant pathogenic fungi.

Conclusion

The present *in-vitro* study demonstrated that natural farming inputs differed considerably in their inhibitory effect against *Macrophomina phaseolina*, causing dry root rot of mungbean. Among the tested inputs, Beejamrit @ 20% recorded the minimum mycelial growth (9.87 mm) and maximum inhibition (89.04%), followed by Jeevamrit (24.17 mm and 73.15%). Cow urine, Neem cake, Ghan Jeevamrit, Ramban and fermented buttermilk showed comparatively lower inhibitory effects. Thus, Beejamrit and Jeevamrit showed considerable antifungal potential against *M. phaseolina* under *in-vitro* conditions, although further validation under field conditions is required.

Acknowledgement

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References

1. Anupriya, Chawla N, Ghasolia MK, Prahlad, Bagri RK, Godika S, *et al.* Management of dry root rot of mungbean caused by *Macrophomina phaseolina* through organic amendments, plant extracts and bio agents, 2024.

2. Fuhlbohlm MJ, Ryley MJ, Aitken EAB. Infection of mungbean seed by *Macrophomina phaseolina* is more likely to result from localized pod infection than from systemic plant infection. *Plant Pathology*,2013;62(6):1271-1284.
3. Gupta GK, Sharma SK, Ramteke R. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). *Journal of Phytopathology*,2012;160(4):167–180.
4. Iqbal U, Mukhtar T. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. *The Scientific World Journal*,2014;2014(1):950175.
5. Nene YL, Thapliyal PN. Fungicides in plant disease control. Oxford & IBH Publishing Co., New Delhi, India, 1979.
6. Palekar S. The philosophy of spiritual farming: Zero budget natural farming. Amravati, Maharashtra, India: Zero Budget Natural Farming Research, Development and Extension Movement, 2006.
7. Prakash N, Sinha AP. *In vitro* studies of inhibitory activity of plant extracts and cow urine on mycelial growth of stem rot, *Sclerotium oryzae* of rice. *J Appl Nat Sci*,2017;9(1):24-8.
8. Rajeswari E, Padmodaya B, Viswanath K, Sumathi P. Anti-fungal effect of organic amendments on the mycelial growth and sclerotial viability of *Rhizoctonia solani* Kuhn. *Andhra Pradesh Journal of Agricultural Sciences*,2018;4(4):259-267.
9. Saharan BS, Tyagi S, Kumar R, Vijay, Om H, Mandal BS, *et al.* Application of Jeevamrit improves soil properties in zero budget natural farming fields. *Agriculture*,2023;13(1):196.
10. Sharma R, Chadak S. Residual soil fertility, nutrient uptake, and yield of okra as affected by bioorganic nutrient sources. *Communications in Soil Science and Plant Analysis*,2022;53(21):2853-2866.
11. Somdutt B, Karan B, Rajendra R, Shekhawat PS. Jeevamrut and panchagavya's consequences on growth, quality and productivity of organically grown crops: A Review. *Agric Rev*, 2021.
12. Sreenivasa MN, Naik N, Bhat SN. Beejamrutha: A source for beneficial bacteria. *Karnataka Journal of Agricultural Sciences*,2009;22(5):1038–1040.
13. Srinivas A, Devi GU, Sagar BV, Srinivas C, Balram M, Mallaiah B. Efficacy of organic soil amendments, fungicides and chitosan against *Macrophomina phaseolina* under *in vitro* conditions, 2021.
14. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*,1947;159(4051):850.