



Impact of Seed-borne Mycoflora on Seed Quality Parameters in Greengram (*Vigna radiata* L.)

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ABSTRACT

Background: Greengram (*Vigna radiata*) is an important pulse crop widely cultivated in tropical and subtropical regions for its nutritional and economic value. However, seed-borne fungal pathogens are a major constraint to its productivity, viability and seed quality. These pathogens infect seeds during various developmental stages, leading to reduced germination rates, lower seed vigor and diminished storage potential. The present study aimed to assess seed-borne fungal infections in greengram genotypes using the agar plate method and to evaluate the efficacy of selected chemical fungicides and biological agents under pot culture conditions.

Methods: A total of twenty-four greengram genotypes were screened for seed-borne fungal infections using the agar plate method. The incidence of infection was recorded and fungal species were isolated and identified based on morphological characteristics. To manage these infections, pot culture experiments were conducted using the most susceptible genotype (DGGV 2). The study evaluated the bio-efficacy of selected chemical fungicides (Sprint and Xelora) and biological (*Trichoderma harzianum*). Seed germination percentage, vigor index, plant growth and yield parameters were recorded and analyzed to assess the effectiveness of the treatments.

Result: Eight seed-borne fungal species were isolated and identified. The genotype TARM 1-28-1 exhibited the lowest infection rate (4.83%) and the genotype DGGV 2 showed the highest infection rate (12.50%). Seed treatment with Sprint and *T. harzianum* in susceptible genotype DGGV 2 significantly reduced fungal incidence and enhanced seedling growth and yield performance. Xelora was particularly effective in controlling anthracnose caused by *Colletotrichum* spp. The study highlights the importance of integrating resistant genotypes with effective seed treatment strategies for managing seed-borne fungal diseases and enhancing greengram productivity.

Key words: Green gram, Mycoflora, Seed quality, Seed treatment, Seedborne diseases.

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilezek] is an important annual pulse crop of the Leguminaceae family, with its origin traced to the Indian subcontinent where domestication dates back to 1500 BC (Darekar *et al.*, 2024). It is ranked as the third most important pulse crop after chickpea and pigeon pea, cultivated during both kharif and summer seasons. In India, during 2020-21, greengram was produced on 4.5 million hectares with a total output of 2.64 million tonnes, accounting for about 10-12% of the national pulse production, with an average productivity of 548 kg ha⁻¹ (Anonymous, 2021). Nutritionally, greengram is highly valued as it contains 24.5% high-quality protein, 1.3% fats, 56.6% carbohydrates and 3% dietary fibre, along with essential minerals such as calcium (140 mg), iron (8.4 mg) and phosphorus (280 mg).

Despite its economic and nutritional importance, greengram productivity is significantly constrained by seed-borne infections. Like other pulses, it is vulnerable to a wide range of pathogens at different growth stages, from seed germination to maturity. Seed-borne microflora represents a major bottleneck in its production, as infections associated with seeds-either externally or internally-can lead to seed rot, seedling blight and substantial reductions in germination (up to 30-40%), ultimately lowering seedling vigour. The crop is reported to be attacked

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by nearly 35 fungal species, in addition to several viral, bacterial and nematode pathogens, all contributing to considerable yield losses (Lal and Singh, 2023).

Ensuring healthy seeds with high germination and genetic purity is, therefore, fundamental for improving both quality and yield. As a prophylactic strategy, resistant genotypes are often employed to suppress the spread of seed-borne pathogens. In addition, seed treatment has emerged as one of the earliest and most widely adopted plant protection measures. The application of fungicidal seed dressings helps safeguard seeds against pathogenic infections that interfere with germination or threaten young seedlings immediately after emergence. Beyond disease suppression, fungicidal treatments may also enhance seed performance by providing growth-promoting substances. Furthermore, the use of bioprotectants has gained attention due to their ability to colonize roots, suppress pathogens and simultaneously stimulate plant growth, offering a sustainable alternative in integrated disease management (Sharma *et al.*, 2014). Thus, the adoption of effective seed health management strategies remains crucial for harnessing the full potential of greengram and sustaining its role as a key dietary and economic pulse crop. Considering the significance of seed health in greengram and the need for effective management of seed-borne pathogens, the present investigation was undertaken to screen the different genotypes for seed borne mycoflora infection and to evaluate the efficacy of seed treatment approaches, including chemical and biological seed treatments.

MATERIALS AND METHODS

Seed materials

The present investigation was carried out during the year 2021-22 at Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Dharwad under laboratory conditions. Twenty-four different mung bean genotypes (Table 1) were collected from AICRP on MULLaRP, MARS, UAS, Dharwad. The genotypes were screened for seed infection by fungal diseases and were subjected to seed health testing by agar plate method.

Screening of genotypes for seed borne mycoflora

Per cent seed infection by different seed borne mycoflora of selected genotypes were assessed under laboratory conditions using standard agar plate method. Based on the per cent seed infection, genotypes were categorized into tolerant and susceptible to seed borne mycoflora.

Identification of seed borne fungi

The agar plate method was used for the detection and identification of microorganisms associated with seed; 20 ml potato dextrose agar (PDA) medium was poured in each petri-plate. Surface sterilization of seeds was done according to the method given by Arnold *et al.* (2000). First, seeds were soaked in 70% (v/v) ethanol for 3 min followed

by 4% (v/v) sodium hypochlorite for 3 min and again with 70% (v/v) ethanol for 3 min and then seeds were washed with sterile double distilled water thrice to remove sterilization solution. The entire sterilization process was performed inside a laminar air flow chamber and the seeds were dried after washing with double distilled water.

Surface-sterilized seeds (25 per plate) were aseptically placed at equal distances on agar medium and incubated at $28\pm1^{\circ}\text{C}$ under 12-hour alternating light and dark conditions. The plates were observed regularly for the emergence of fungal colonies. Once growth was detected, individual colonies were subcultured to obtain pure isolates. Fungal species were identified based on spore morphology and colony characteristics, following the descriptions provided by Ellis (1971) and Barnett and Hunter (1972).

Evaluation of seed quality parameters

The effect of pathogenic seed borne mycoflora from seeds of greengram varieties on seed germination and seedling vigour index was studied by adopting the procedure of between paper (roll paper towel) method as described by ISTA's rules, 2019. Randomly selected 400 seeds were placed on moist paper towels in 4 replications at equidistance and incubated it for seven days at room temperature and then examined the normal, abnormal seedlings and ungerminated seeds by naked eye and presence of mycoflora observed by stereo-binocular microscope (40X magnification).

Table 1: List of greengram genotypes used in the study.

Genotype	Pedigree
DGWW 16	DGGV2 × WGG 42
DGWW 4	DGGV2 × WGG 42
DGWW 31	DGGV2 × WGG 42
DGG-182-1	IPM 2-14 × AKM-9904
DGG 215-2	IPM 2-14 × IPM 2-17 (2)
DGGV 79-2	Mutant of IPM 2-17 (22-1-1)
DGG 301	-
DGGV 2X IPM 410-3	DGGV 2 × IPM 410-3
DGWW 44	DGGV2 × WGG 42
DGWW 53	-
DGG 20	BGS 9 × TM 96-2 (GG 20-4)
DGG 51	-
DGWW 30	DGGV2 × WGG 42
DGWW 86	DGGV2 × WGG 42
DGG 21	BGS 9 × TM 96-2 (GG 19-1)
Sonamung 44-2	DGG 75
MBRD 56	DGGV 7 × V-02-704
MBRD 58	DGGV 7 × V-02-802
DGG 82	Mutant of Vamban-2 (21-2)
DGG 152	-
TARM 1-28-1	DGG 96
BGS 9 X AKM 9904	DGG 182-1
DGG 1	-
DGGV 2	China mung × TM 98-50

Pot culture evaluation of seed treatments for management of seed-borne fungal diseases

Based on the susceptibility to seed borne diseases viz., *Cercospora* and anthracnose, DGGV 2 genotype had been used to evaluate different seed- dressing fungicides and bioagents for managing seed-borne fungal diseases. A total of seven treatments were formulated to test the efficacy of the seed treatment materials against cercospora leaf spot and anthracnose diseases. Each treatment had five replications with four plants per pot. Standard agronomic practices were followed uniformly. Treatment details:- T₁: Control, T₂: Seed dressed with Carbendazim 50% WP @ 2 g/kg seeds, T₃: Seed dressed with Mancozeb 50 % WP+ Carbendazim 25% WS (Sprint) @ 2 g/kg seeds, T₄: Seed dressed with Thiophanate methyl + pyraclostrobin (Xelora) @ 2 g/kg seeds, T₅: Seed dressed with *Tricoderma harzianum* @ 10g/kg seeds, T₆: Seed dressed with *Pseudomonas fluroscens* @ 10 g/kg seeds, T₇: Seed dressed with *Bacillus subtilis* @ 10 g/kg seeds.

Disease severity (PDI) of cercospora leaf spot and anthracnose were assessed at 45 and 65 DAS using standard rating scales (0-9 for *Cercospora*, Naresh *et al.* (2020); 0-5 for anthracnose Rajkumar and Mukhopadhyay (1986).

$$PDI = \frac{\text{Sum of ratings}}{\text{Total leaves observed} \times \text{Maximum score}} \times 100$$

Growth parameters viz plant height, number of branches and chlorophyll content (SPAD) were recorded at 45 and 65 DAS. Yield parameters viz number of pods per plant, pod dry weight (g), seeds per pod, seed yield per plant (g) and seed test weight were measured at harvest.

RESULTS AND DISCUSSION

Evaluation of seed health and quality parameters

Based on seed health testing we could identify a total of eight seed borne fungi (Fig 1). The results of the study revealed the dominance of *Aspergillus flavus* (22.08%), *Aspergillus*

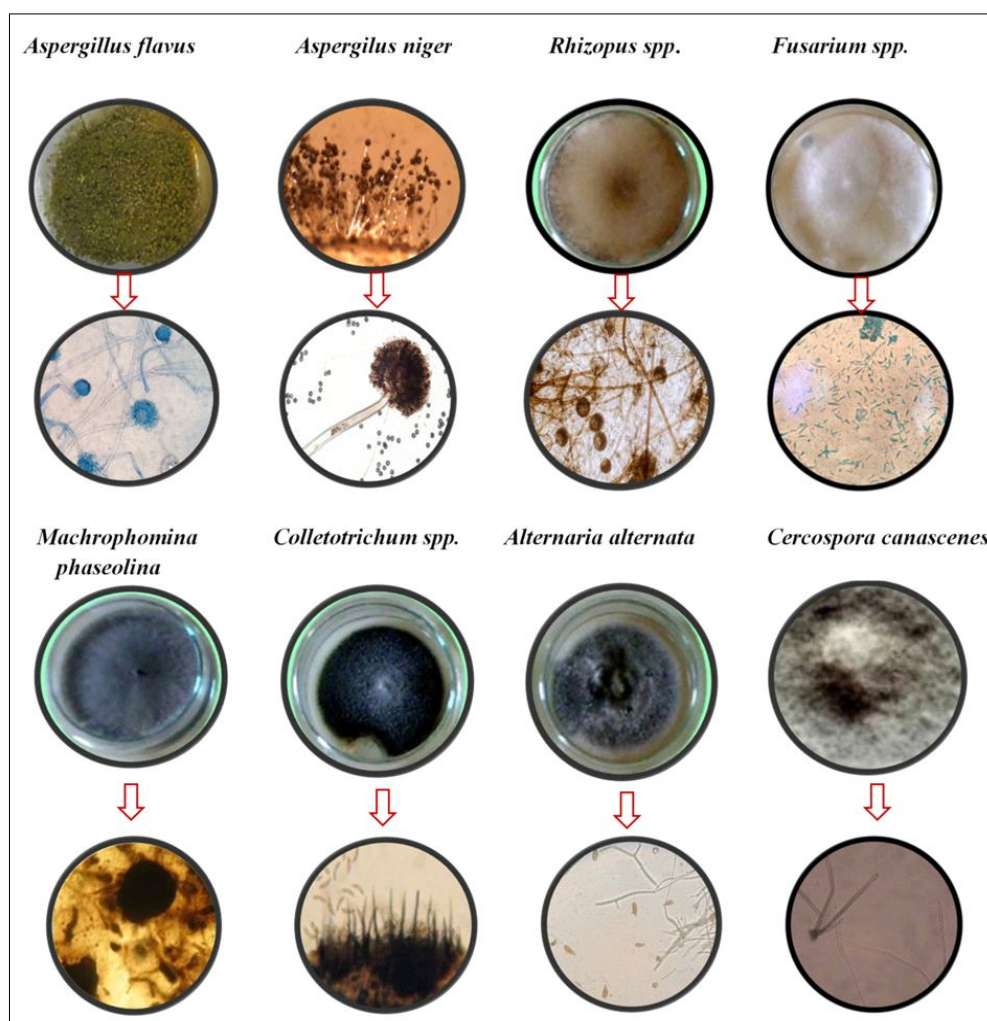


Fig 1: Morphological characteristics of mycoflora isolated from greengram genotypes (Front view and microscopic view (40X Magnification).

niger (16.94%), *Macrophomina phaseolina* (5.83%), *Rhizopus spp* (5.77%), *Colletotrichum sp.* (5.14%), *Fusarium* (4.44%), *Alternaria alternata* (4.39%) and *Cercospora canascens* (3.83%) (Table 2). Genotype DGGV 152 showed the highest percent seed infection (12.50%), while TARM 1-28-1 recorded the lowest (4.83%) percent infection. Similar experiments with different genotypes were conducted by Tandel (2004) who screened different greengram varieties for several seed fungi including *Machrophomina phaseolina*, *Curvularia sp.*, *Aspergillus sp.*, *Alternaria sp.*, *Rhizopus sp.*, *Colletotrichum spp* using moist blotter and PDA plate methods and found GM-3 has the highest, followed by BARC TM 96-2. Dolas (2018) noticed seed borne mycoflora associated with the seeds of

greengram varieties viz., BPMR-145, Utkarsh, Kopergaon, BM-2002-1, AKM-8802, DGGV-2, SML 668 and Vaibhav.

In case of seed quality parameters, genotype TARM 1-28-1 recorded highest germination percentage (89%) and seedling vigor index (3355) with lowest seed infection (5.33%) and electrical conductivity (0.18 dS/m). In contrast, the genotype DGGV 152 had the highest seed infection of 23.67% and electrical conductivity (0.58 dS/m) with lowest germination percentage (65.33%) and seedling vigor index (1194) (Fig 2 and 3). With this data the genotype TARM 1-28-1 found to be tolerant, while DGGV 152 genotype was susceptible (Fig 4). The reduced seed quality of susceptible genotype was the result of increased infection by the mycoflora which makes seed coat permeability a

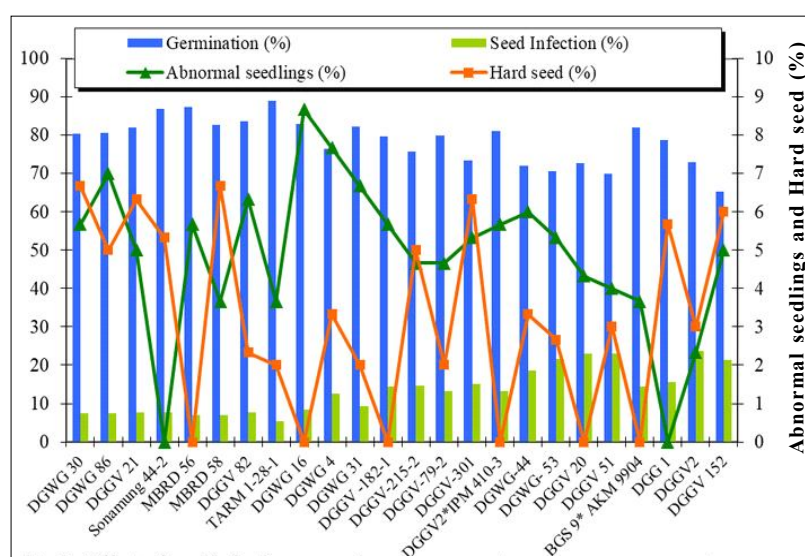


Fig 2: Effect of seed infection on seed quality parameters of greengram. genotypes.

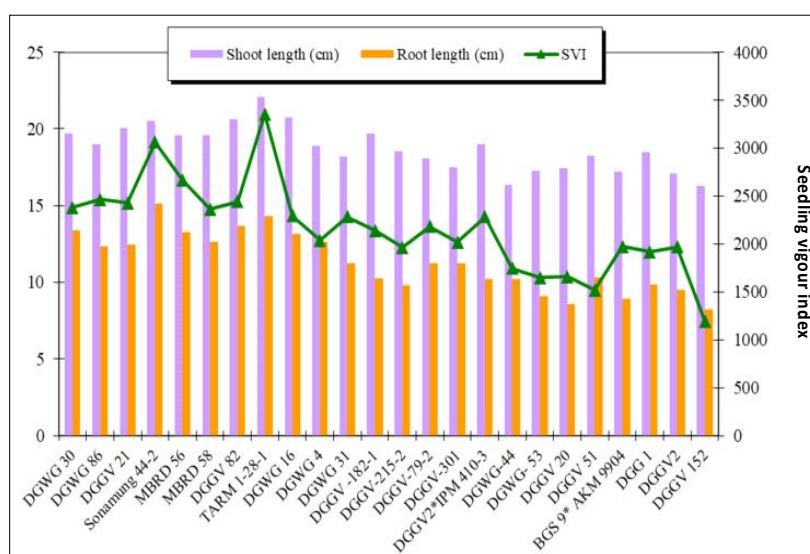


Fig 3: Effect of seed infection shoot length, root length and SVI of greengram genotypes.

Table 2: Per cent seed infection of different genotypes of greengram by agar plate method.

Genotypes	<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i>	<i>Fusarium sp.</i>	<i>Alternaria sp.</i>	<i>Colletotrichum sp.</i>	<i>M. phaseolina</i>	<i>Cercospora sp.</i>	Mean
DGWG30	12.67 (3.63)	9.33 (3.12)	8.00 (2.92)	3.67 (2.04)	5.33 (2.39)	2.67 (1.65)	10.67 (3.33)	4.33 (2.20)	7.08
DGWG86	13.33 (3.71)	9.33 (3.12)	9.33 (3.12)	3.67 (2.02)	6.67 (2.65)	8.00 (2.92)	9.33 (3.12)	3.00 (1.86)	7.83
DGGV 21	13.33 (3.71)	9.33 (3.13)	0.00 (0.71)	3.33 (1.95)	6.67 (2.65)	5.33 (2.18)	3.33 (1.73)	3.33 (1.95)	5.58
Sonamung 44-2	12.00 (3.50)	0.00 (0.71)	0.00 (0.71)	5.33 (2.41)	9.33 (3.12)	4.00 (1.91)	5.33 (2.17)	6.67 (2.68)	5.33
MBRD 56	13.33 (3.71)	9.33 (3.12)	13.33 (3.71)	2.67 (1.64)	5.33 (2.39)	4.00 (1.91)	10.67 (3.34)	2.67 (1.64)	7.67
MBRD 58	4.67 (2.06)	6.67 (2.65)	6.67 (2.65)	4.67 (2.26)	10.67 (3.33)	5.33 (2.18)	4.00 (2.08)	6.00 (2.53)	6.09
DGGV 82	5.33 (2.18)	12.00 (3.50)	0.00 (0.71)	4.67 (2.26)	0.00 (0.71)	6.67 (2.65)	9.33 (3.06)	6.00 (2.53)	5.50
TARM 1-28-1	9.33 (3.12)	12.00 (3.54)	8.00 (2.92)	2.00 (1.47)	0.00 (0.71)	4.00 (1.91)	0.00 (0.71)	3.33 (1.79)	4.83
DGWG 16	13.33 (3.71)	12.00 (3.54)	9.33 (3.12)	5.00 (2.30)	5.33 (2.18)	8.00 (2.92)	6.67 (2.65)	5.67 (2.46)	8.17
DGWG 4	10.67 (3.33)	13.33 (3.71)	12.00 (3.50)	4.67 (2.27)	8.00 (2.92)	8.00 (2.92)	8.00 (2.92)	4.67 (2.24)	8.67
DGWG31	17.33 (4.22)	12.00 (3.54)	12.00 (3.50)	6.00 (2.55)	0.00 (0.71)	8.00 (2.92)	10.67 (3.33)	4.67 (2.23)	8.83
DGGV -182-1	18.67 (4.37)	18.67 (4.37)	13.33 (3.71)	0.00 (0.71)	9.33 (3.12)	10.67 (3.33)	0.00 (0.71)	0.00 (0.71)	8.83
DGGV-215-2	22.67 (4.81)	18.67 (4.37)	13.33 (3.71)	7.67 (2.85)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	7.00 (2.73)	8.67
DGGV-79-2	21.33 (4.67)	18.67 (4.37)	0.00 (0.71)	7.33 (2.78)	8.00 (2.92)	0.00 (0.71)	14.67 (3.89)	7.33 (2.80)	9.67
DGGV-301	22.67 (4.81)	21.33 (4.67)	13.33 (3.71)	2.67 (1.44)	0.00 (0.71)	6.67 (2.39)	7.33 (2.79)	0.00 (0.71)	9.25
DGGV2*IPM 410-3	26.67 (5.21)	21.33 (4.67)	0.00 (0.71)	9.33 (3.06)	0.00 (0.71)	4.67 (2.21)	0.00 (0.71)	1.33 (1.18)	7.92
DGWG-44	30.67 (5.58)	30.67 (5.58)	0.00 (0.71)	0.00 (0.71)	2.67 (1.44)	5.33 (2.18)	6.67 (2.65)	0.00 (0.71)	9.50
DGWG-53	36.00 (6.04)	22.67 (4.78)	0.00 (0.71)	8.67 (3.02)	9.33 (3.12)	0.00 (0.71)	0.00 (0.71)	6.00 (2.54)	10.33
DGGV 20	39.33 (6.31)	28.00(5.33)	0.00 (0.71)	9.33 (3.12)	0.00 (0.71)	9.33 (3.12)	0.00 (0.71)	0.00 (0.71)	10.75
DGGV 51	42.67 (6.57)	33.33 (5.81)	6.67 (2.65)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	6.67 (2.65)	0.00 (0.71)	11.17
BGS 9* AKM 9904	28.67 (5.40)	38.67 (6.26)	1.33 (1.18)	6.00 (2.53)	4.00 (1.65)	0.00 (0.71)	2.67 (1.76)	6.00 (2.53)	10.92
DGG 1	42.00 (6.52)	17.33 (4.20)	0.00 (0.71)	4.00 (1.93)	0.00 (0.71)	4.00 (1.65)	5.33 (1.83)	5.33 (2.18)	9.75
DGGV2	26.67 (5.21)	14.67 (3.87)	0.00 (0.71)	0.67 (1.00)	14.67 (3.89)	13.33 (3.71)	5.33 (2.18)	8.67 (3.02)	10.50
DGGV 152	47.67 (6.87)	17.33 (4.22)	12.00 (3.54)	5.33 (2.18)	0.00 (0.71)	5.33 (2.18)	13.33 (3.71)	0.00 (0.71)	12.50
Mean (%)	22.08	16.94	5.77	4.44	4.39	5.14	5.83	3.83	
S.E.m±	0.25	0.20	0.18	0.33	0.32	0.48	0.38	0.27	
CD @ 1%	0.96	0.76	0.69	1.25	1.21	1.83	1.46	1.03	

(Values in parenthesis are sqrt transformed values).

detrimental factor. This is supported by the findings of Mandhare *et al.* (2009) reported reduction of seed germination with seed borne infection of *M. phaseolina* in soybean seeds. Kamble *et al.* (2014) who investigated association of seed borne fungi of greengram and subsequently assessed their effect on germination of seeds and reported that the reduction in seed germination was observed due to seed borne fungi.

Correlation of fungal infection on seed quality parameters in green gram genotypes

Specifically, *A. flavus* exhibited the strongest negative correlations with germination (-0.77**), shoot length (-0.75**), root length (-0.81**) and SVI (-0.82**) followed by *A. niger*, which also significantly reduced these parameters (Table 3). These findings indicate that these two fungal species are highly detrimental to seed health and vigour. Parashar *et al.* (2019) isolated *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Drechslera tetramera*, *Fusarium moniliforme* and *Rhizopus stolonifer* from four pulses, namely chickpea, greengram, pigeon pea and lentil and correlated that these fungi caused reduction in germination percentage and seedling vigour in pulse seeds.

Electrical conductivity (EC), a measure of seed deterioration, showed significant positive correlations with *A. flavus* (0.69**) and *A. niger* (0.47*) further supporting their negative impact on seed quality. Other fungi such as *Rhizopus*, *Fusarium*, *Alternaria*, *Colletotrichum*, *M. phaseolina* and *Cercospora* exhibited weak or negligible correlations with most seed quality parameters indicating minimal

influence. Notably, *Rhizopus* showed a moderate positive correlation with abnormal seedlings (0.33) and *M. phaseolina* had a slight positive correlation with hard seeds (0.24) though these were not statistically significant.

Effect of seed dressing chemicals and bio-agents on seed borne fungal infection in greengram

Effectiveness of various seed treatment materials was evaluated to reduce the infection of cercospora leaf spot caused by *Cercospora sp.* and anthracnose caused by *Colletotrichum sp.* Per cent disease index (PDI) of cercospora leaf spot and anthracnose under pot culture conditions was recorded at 45 and 65 days after sowing (DAS) under pot culture conditions (Table 4). In case of Cercospora leaf spot disease, the untreated control (T_1) consistently showed the highest PDI at both 45 DAS (17.00%) and 65 DAS (27.06%), reflecting natural disease progression in the absence of any protective treatment.

Among the treatments, T_3 (Seed dressed with Mancozeb 50% WP+ Carbendazim 25% WS (Sprint)) found to be the most effective, recording the lowest PDI values of 5.07% at 45 DAS and 10.26% at 65 DAS (Fig 5 and 6). This demonstrates superior protective efficacy and prolonged suppression of Cercospora leaf spot, likely due to stronger residual or systemic activity (Prasad *et al.*, 2024). T_4 (Seed dressed with Thiophanate methyl + pyraclostrobin (Xelora) and T_5 (biological agent Trichoderma harzianum) also showedstley substantial control, with final PDI values at 65 DAS of 12.93% and 10.13%, respectively. This is significant, especially for T_5 (Seed dressed with Tricoderma harzianum),



Fig 4: Effect of seed infection on seed germination potential in resistant (TARM 1-28-1) and susceptible (DGGV 152) genotypes.

Table 3: Correlation of different fungi on seed quality parameters of greengram genotypes.

	<i>A. flavus</i>	<i>A. niger</i>	<i>Rhizopus</i>	<i>Fusarium</i>	<i>Alternaria</i>	<i>Colletotrichum</i>	<i>M. phaseolina</i>	<i>Cercospora</i>
Germination (%)	-0.77**	-0.55**	-0.12	0.00	-0.12	-0.01	-0.04	-0.35
Abnormal seedlings (%)	-0.30	-0.20	0.33	0.05	-0.10	0.20	0.24	-0.25
Hard seed (%)	-0.05	-0.38	-0.04	-0.16	0.06	-0.12	0.24	0.09
Shoot length (cm)	-0.75**	-0.66**	-0.17	-0.20	-0.14	-0.17	-0.15	-0.22
Root length (cm)	-0.81**	-0.67**	-0.27	-0.26	-0.08	-0.04	-0.22	-0.14
SVI	-0.82**	-0.67**	-0.19	-0.16	-0.12	-0.06	-0.02	-0.26
EC (dSm ⁻¹)	0.69**	0.47*	0.13	0.06	0.12	0.03	0.19	0.34

*Correlation is significant at $p \leq 0.05$ level. **Correlation is significant at $p \leq 0.01$ level.

which matches chemical fungicides in performance, highlighting the potential of biocontrol agents in integrated disease management. Biological treatments like T_6 (*Pseudomonas fluorescens*) and T_7 (*Bacillus subtilis*) showed intermediate results with moderate suppression. Their effectiveness could be attributed to induced systemic

resistance or antagonistic activity, although they did not match the top-performing fungicides.

A similar trend was observed for Anthracnose disease, where the control treatment (T_1) had the highest disease incidence with PDIs of 13.00% at 45 DAS and 17.34% at 65 DAS. Here, T_4 (Thiophanate methyl + pyraclostrobin (Xelora)



Fig 5: Infected seedlings showing symptoms of cercospora leaf spot and anthracnose (45 DAS).

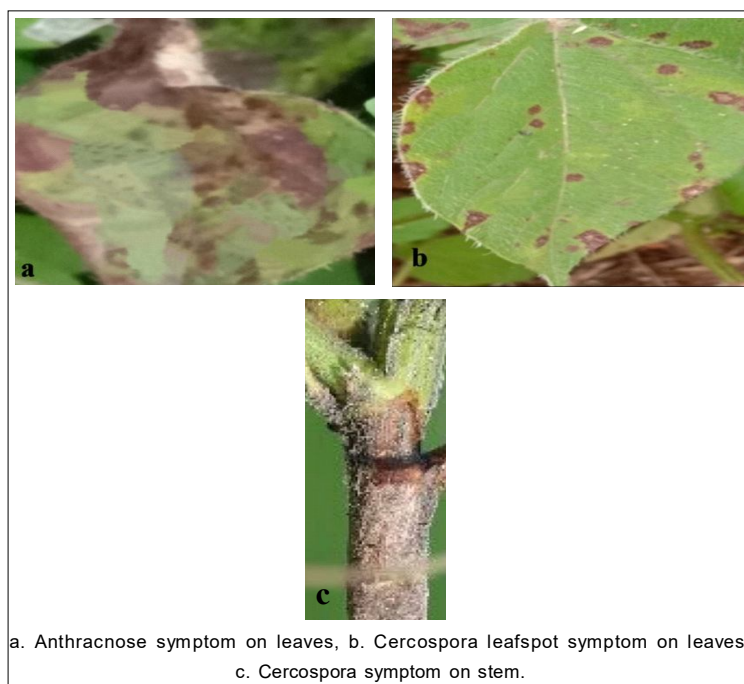


Fig 6: Disease symptoms of anthracnose and *Cercospora* leaf spot.

proved most effective, reducing PDI to 5.07% at 45 DAS and 8.60% at 65 DAS, followed closely by T₅ (*Trichoderma harzianum*) i.e., 5.66% and 8.34% respectively. The biological efficacy of *T. harzianum* is particularly remarkable as it not only suppresses disease efficiently but also promotes plant health through rhizosphere colonization and competition with pathogens. Gveroska *et al.* (2012) reported that volatile compounds and inhibitory enzymes like chitinase are released by *Trichoderma harzianum*, which diffuse through seed pores and cause deformations of chitin and also cause deformations of hyphae and thus reduces its infection. T₂ (Carbendazim 50 % WP) and T₃ [Mancozeb 50% WP+ Carbendazim 25% WS (Sprint)] also performed well but were marginally less effective than T₄ and T₅ for Anthracnose control. Similar findings reported by Deshmukh *et al.* (2016) in greengram that PDI of anthracnose was significantly lower in dry seed treatment with Carbendazim + Mancozeb as compared to other treatments. Purushotham *et al.* (2025) reported that seed treatment of carbendazim 12% + mancozeb 63% @ 3.0 g/kg seed and mancozeb 75 WP @ 3.0 g/kg seed of greengram had the lowest anthracnose disease index compared to control under glass house conditions.

Effect on seed treatment on plant growth and yield attributes

Seed treatment had a marked influence on plant height at both 45 and 65 days after sowing (DAS) (Table 5). Treatment T₅ recorded the maximum plant height (44.03 cm at 45 DAS and 46.46 cm at 65 DAS), significantly higher than the control (T₁), which had the lowest values (37.19 cm and 40.88 cm, respectively). The differences were statistically significant (CD at 1% = 2.94 at 45 DAS and 1.50 at 65 DAS), indicating the positive influence of T₅ on vegetative growth.

The number of branches per plant followed a similar trend, with T₅ exhibiting the highest values (5.25 at 45 DAS and 16.00 at 60 DAS), significantly surpassing T₁ (4.20 and 10.80, respectively). Enhanced branching is often correlated with improved assimilate partitioning and better pod development. This is more or less similar to the findings of Pradhan *et al.* (2017) in pot culture experiment of greengram.

Similarly, Chlorophyll content, measured using SPAD readings, was highest in T₅ (19.25 at 45 DAS and 16.00 at 60 DAS), reflecting improved physiological activity and photosynthetic efficiency. In contrast, T₁ recorded the lowest SPAD values (13.65 and 10.80), suggesting limited chlorophyll accumulation and reduced photosynthetic potential. Saurabh *et al.* (2020) reported that mycoflora

Table 4: Effect of seed treatments on per cent disease index (PDI) of *Cercospora* leaf spot and anthracnose at 45 and 65 DAS under pot culture conditions.

Treatments	Cercospora leaf spot		Anthracnose	
	45 DAS	65 DAS	45 DAS	65 DAS
T ₁	17.00 (24.35)*	27.06 (31.35)*	13.00 (21.13)*	17.34 (24.60)*
T ₂	8.33 (16.77)	18.13 (25.19)	7.67 (16.07)	11.00 (19.36)
T ₃	5.07 (12.98)	10.26 (18.67)	6.13 (14.29)	11.33 (19.67)
T ₄	6.87 (15.19)	12.93 (21.06)	5.07 (13.00)	8.60 (17.05)
T ₅	7.20 (15.56)	10.13 (18.54)	5.66 (13.76)	8.34 (16.77)
T ₆	7.67 (16.07)	17.67 (24.84)	6.26 (14.49)	9.00 (17.45)
T ₇	8.40 (16.85)	18.90 (25.83)	7.00 (15.33)	9.67 (18.11)
S.Em (±)	0.23	0.41	0.30	0.26
CD (p<0.01)	0.93	1.61	1.19	1.02

Table 5: Effect of seed treatments on growth and yield parameters of greengram.

Treatments	Plant height (cm)		No. of branches/plant		Chlorophyll content (Spad reading)		No. of pods/plant		SYP (g)	TW (100 seeds) (g)
	45 DAS	65 DAS	45 DAS	60DAS	45 DAS	60DAS	60DAS	65 DAS		
T ₁	37.19	40.88	4.2	10.80	13.65	10.80	10.80	32.82	6.65	4.22
T ₂	41.48	43.77	4.7	15.25	18.90	15.25	15.25	36.35	10.13	4.42
T ₃	43.00	45.58	5.15	15.90	20.35	15.90	15.90	37.94	10.73	4.49
T ₄	43.08	45.85	4.75	15.40	19.05	15.40	15.40	35.86	9.57	4.43
T ₅	44.03	46.46	5.25	16.00	19.25	16.00	16.00	38.72	10.18	4.52
T ₆	43.78	46.00	4.6	13.75	18.80	13.75	13.75	36.54	8.46	4.43
T ₇	42.58	44.00	4.65	12.95	17.80	12.95	12.95	33.94	8.34	4.34
S.Em (±)	0.75	0.38	0.09	0.48	0.61	0.48	0.48	0.58	0.30	0.06
CD (p<0.01)	2.94	1.50	0.37	1.91	2.41	1.91	1.91	1.30	1.19	0.12

SYP: Seed yield per plant; TW: Test weight.

Table 6: Correlation of cercospora leaf spot and anthracnose with growth and yield parameters of greengram genotype DGGV 2 treated with different fungicides and bio agents.

	Cercospora	Anthracnose	Plant Ht	No. of branches	Chlorophyll	No. of pods	Pod w t	No. of seeds	Seed yield	Test weight
Cercospora	1									
Anthracnose	0.82*	1								
Plant height	-0.88**	-0.85*	1							
Number of branches	-0.61	-0.40	0.21	1						
Chlorophyll	-0.76*	-0.55	0.62	0.68	1					
Number of pods	-0.87*	-0.92**	0.81*	0.58	0.58	1				
Pod weight	-0.88**	-0.92**	0.79*	0.65	0.65	0.99**	1			
Number of seeds	-0.68	-0.56	0.32	0.97**	0.66	0.73	0.78*	1		
Seed yield	-0.86*	-0.76*	0.58	0.88**	0.77*	0.86*	0.91**	0.95**	1	
Test weight	-0.75*	-0.87*	0.70	0.62	0.74	0.91**	0.93**	0.75	0.87*	1

*Correlation is significant at $p \leq 0.05$ level. **Correlation is significant at $p \leq 0.01$ level.

inhibits seed germination, reduces biomass of seedlings and also total chlorophyll content of leaves.

A significant increase in the number of pods per plant was observed under T_5 (38.72), which was markedly higher than T_1 (32.82). The improvement in pod number under T_5 could be attributed to better vegetative growth and nutrient uptake efficiency facilitated by the seed treatment. Seed yield per plant was significantly influenced by the treatments, with T_5 producing the highest yield (10.18 g), followed by T_3 (10.73 g). Similarly, T_5 also showed the greatest 100-seed weight (4.52 g), indicating better seed development and filling. These results underscore the positive effect of T_5 on yield enhancement, supported by statistically significant differences (CD at 1% = 1.19 for yield and 0.12 for seed weight). Negi *et al.* (2021) found that seed biopriming of French bean with *Trichoderma viridae*+ PGPR strain significantly improved plant growth, pod yield, reduced disease incidence and seed yield.

Among all treatments, T_5 consistently outperformed others across all measured parameters, indicating its superior efficacy in promoting growth and yield of greengram. The control treatment (T_1) consistently recorded the lowest values, highlighting the critical role of appropriate seed treatments in improving crop performance. Deshmukh *et al.* (2020) reported that dry seed treatment with either mix formulation of Carbendazim+ Mancozeb or Thiophanate methyl or Carbendazim @ 2.5 g kg⁻¹ seeds is very effective in field to get maximum seed germination, better plant growth, root growth, yield parameters and yield of greengram. Sharma and Roy (2021) also found the similar findings of seeds treated with combined inoculation of different bio agents recorded significantly higher response in terms of reduction in percentage of cercospora leaf spot occurrence in greengram.

All these parameters are negatively correlated with cercospora leaf spot and anthracnose which reduces plant growth, chlorophyll content due to which photosynthetic activity is affected, which affects reduction in number of pods per plant and leads to reduction in test seed yield (Table 6). Due to seed treatment with different fungicides

and bio agents, there will be decrease in these negative effects on growth parameters and yield parameters by counteracting seed borne diseases i.e., cercospora leaf spot and anthracnose in greengram.

Mancozeb 50 % WP+ carbendazim 25% WS (Sprint) seed dressing fungicide found more effective in both invitro and insitu in controlling seed borne mycoflora and influences different seed quality parameters as it is the comby product consists of both contact and systemic fungicide. Bio agent *Trichoderma harzianum* acts against seed borne mycoflora by mycoparasitism and antagonistic mode of action, Pradhan *et al.* (2017).

CONCLUSION

Eight seed borne fungi viz., *Fusarium oxysporum*, *Macrophomina phaseolina*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus spp*, *Colletotrichum spp*, *Alternaria alternata* and *Cercospora canacens* were found associated with seeds of greengram genotypes. DGGV 152 found the most susceptible genotype and TARM 1-28-1 found to be tolerant to seed borne fungal infections. Seed borne fungi were found pathogenic to seeds of greengram resulting in reduction in seed germination and seedling vigour index. The seed borne mycoflora viz., *Aspergillus flavus* and *Aspergillus niger* were found more damaging and reduces seed quality parameters. The seed treatment with Mancozeb 50% WP+Carbendazim 25% WS (Sprint) and bioagent *Trichoderma harzianum* exhibited lowest seed mycoflora, enhanced seed germination and seedling vigour index in naturally infected seed both in lab and pot culture conditions.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

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REFERENCES

- Anonymous, (2021). Area, Production and Productivity of Greengram. www.desagri.gov.in.
- Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.A. (2000). Are tropical fungal endophytes hyperdiverse? *Ecology Letters*. **3**: 267-274.
- Barnet, H.L., Hunter, B.B. (1972). Illustrated Genera of Imperfect Fungi. APS Press. U.S.A. 273.
- Darekar, S.G., Sontakke, P.L., Sarode, S.B. and Kal, H.P. (2024). *In vitro* evaluation of fungicides against *Cercospora canescens* causing leaf spot of green gram. *Journal of Plant Disease Sciences*. **19(1)**: 93-95.
- Deshmukh, M.A., Gade, R.M., Belkar, Y.K. and Koche, M.D. (2016). Efficacy of bioagents, biofertilizers and soil amendments to manage root rot in greengram. *Legume Research-An International Journal*. doi: 10.18805/lr.v0i0F.6772.
- Deshmukh, A.J., Sabalpara, A.N. and Bambharolia, R.P. (2020). Efficacy of fungicidal seed treatments on seed borne diseases of greengram. *International Journal of Economic Plants*. **7(3)**: 138-143.
- Dolas, R.M., Gawade, S.B. and Kasture, M.C. (2018). Efficacy of seed treatment of fungicides, bio agents and botanicals on seed mycoflora, seed germination and seedling vigour index of mung bean. *Journal of Pharmacognosy and Phytochemistry*. **7(5)**: 1074-1077.
- Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, 608. <https://doi.org/10.1079/9780851986180.0000>.
- Gveroska, B. and Ziberoski, J. (2012). *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. *ATI-Applied Technologies and Innovations*. **7(2)**: 67-76.
- Kamble, G., Giri, G.K. and Patil, A.N. (2014). Detection of seed borne fungi in mung bean from rain affected seed. *Journal of Plant Disease Sciences*. **9(1)**: 91-93.
- Lal, M.L. and Singh, D. (2023). Seed mycoflora of green gram. *Madras Agricultural Journal*. **84**: 681-685.
- Mandhare, V.K., Gawade, S.B. and Suryawanshi, A.V. (2009). Detection and transmission of seed borne infection of *Macrophomina phaseolina* causing charcoal rot in soybean. *Journal of Plant Diseases and Protection*. **4(1)**: 130-131.
- Naresh, K., Sunil, K., Satyadev, P. and Shivam, M. (2020). Cercospora leaf spot disease of green gram and its management: A review. *Journal of pharmacognosy and phytochemistry*. **9**: 1574-1576.
- Negi, S., Bharat, N.K. and Kumar, M. (2021). Effect of seed biopriming with indigenous PGPR, *Rhizobia* and *Trichoderma* sp. on growth, seed yield and incidence of diseases in French bean (*Phaseolus vulgaris* L.). *Legume Research-An International Journal*. **44(5)**: 593-601. doi: 10.18805/LR-4135.
- Parashar, R., Rizvi, G. and Sinha, P. (2019). Seed mycoflora of some pulses collected from Bundelkhand region. *Journal of Pharmacognosy and Phytochemistry*. **8(3)**: 1981-1985.
- Pradhan, S., Lakpale, N., Tiwari, P. and Pradhan, A. (2017). Effect of seed treatment and seed borne mycoflora on vigour of mung bean [*Vigna radiata* (L.) Wilczek] grown in agro-climatic zones of Chhattisgarh, India. *International Journal of Current Microbiology and Applied Sciences*. **6(11)**: 1946-1954.
- Prasad, D., Gupta, K. and Singh, V.P. (2024). Management of cercospora leaf spot of mungbean [*Vigna radiata* (L.) Wilczek] using fungicides and host resistance in Bundelkhand Region of Uttar Pradesh, India. *Legume Research-An International Journal*. **47(2)**: 318-322. doi: 10.18805/LR-4641.
- Purushotham, P., Rakholiya, K.B. and Vanani, K.D. (2025). Effectiveness of fungicides against *Colletotrichum lindemuthianum* causing anthracnose of green gram [*Vigna radiata* (L.) Wilczek]. *Legume Research: An International Journal*. **48(8)**: 1361-1368. doi: 10.18805/LR-5137.
- Rajkumar and Mukhopadhyay, A.N. (1986). Field evaluation of urdbean germplasm lines against *Colletotrichum capsici*. *Indian Journal of Mycology and plant pathology*. **17**: 66.
- Sharma, P., Sharma, M., Raja, M. and Shanmugam, V. (2014). Status of *Trichoderma* research in India: A review. *Indian Phytopathology*. **67(1)**: 1-19.
- Sharma, P. and Roy, M. (2021). Increase in defense response of biocontrol agents for reduction of cercospora leaf spot of greengram (*Vigna radiata* L.). *Journal of Pharmacognosy and Phytochemistry*. **10(1)**: 1480-1483.
- Saurabh, N. and Singh, N.K. (2020). Study of impact of seed borne fungal pathogen on seed germination, seedling biomass and chlorophyll contents of four different pulse crops. *Indian Journal of Scientific Research*. **11(1)**: 125-131.
- Tandel, D.H. (2004). Epidemiology and management of leaf blight of greengram (*Phaseolus aureus* Roxb) caused by *Macrophomina phaseolina* (Tassi) Goid. M.Sc. (Agri.) Thesis, Navsari Agriculture University, Gujrat.