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Prospecting of Bioagents and Screening of Varieties against Anthracnose of Green Gram

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Anthracnose poses a significant threat to green gram cultivation in India. This study focused on evaluating various bioagents and cultivars efficacy in combating *Collectotrichum lindemuthianum* through *in vitro* bioassays and glasshouse investigations.
Study Design: Dual culture technique and screening of varieties
Place and Duration of Study: The laboratory studies were conducted in the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India during 2020–2021.
Methodology: The dual culture method was employed to gauge the potency of biocontrol agents, while variety screening helped to identify resistant and susceptible green gram varieties against *C. lindemuthianum.*

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Results: *In vitro* assessments revealed that among the five biocontrol agents tested, *Trichoderma virens* (87.63%) and *T. viride* (85.41%) exhibited significant suppression of mycelial growth compared to the untreated control. However, in greenhouse pot culture experiments involving eight different genotypes, none were found to be immune to green gram anthracnose. Nevertheless, Pusa 1431, KM-2328, TARM-18, and GM 6 exhibited a resistant response against anthracnose, while BPMR-145 and Vaibhav proved susceptible to the disease.
Conclusion: Biocontrol agents are cost-effective and safe for disease management, while integrating resistant varieties is a solid strategy. These high-yielding, environmentally safe, and economically viable methods benefit farmers. Recognizing resistant genotypes is crucial for breeding disease-resistant varieties, as they limit field spread and reduce conidia production of *C. lindemuthianum* causing green gram anthracnose.

Keywords: Anthracnose; bioagents; Collectotrichum lindemuthianum; dual culture; green gram; variety.

1. INTRODUCTION

Green gram, scientifically known as Green gram [*Vigna radiata* (L.) Wilczek, Syn.: *Phaseolus aureus*Roxb., *Phaseolus radiates* L.], is the third most significant pulse crop in India, following bengal gram and red gram. Renowned by various names like phaseolus bean, mung, or mung bean, it holds a pivotal place in Indian agriculture. This versatile crop isn't just cultivated for its seeds but also for green manure and fodder. Its moniker "Golden Bean" is attributed to its exceptional nutritional value and its role in enriching soil nitrogen levels, making it highly regarded [1]. In India, mungbean cultivated across 2.37 million hectares of land with total grain production of 20.89 million tonnes [2]. Its adaptability to various cropping systems is matched by its nutritional richness, boasting protein content ranging from 25% to 28%, 1.0% to 1.5% oil, 3.5% to 4.5% fiber, 4.5% to 5.5% ash, and 62% to 65% carbohydrates on a dry weight basis. This legume's high digestibility makes it a favored choice for infants, recovering patients, and the elderly [3]. Interestingly, unlike some other pulses, it doesn't induce flatulence. Beyond its significance in human diets, it is an essential component of increasing soil fertility by fixing atmospheric nitrogen, contributes to its importance in sustainable agricultural practices [4].

Green gram, an annual legume belonging to the Fabaceae family, is an autogamous diploid plant (2n=22). Mung bean typically grows as an erect or semi-erect plant, attaining heights between 30 to 160 cm. It boasts a well-developed root system and features alternate trifoliate leaflets. Its flowers, colored yellow or greenish, exhibit a papillonaceous structure. The plant bears long, cylindrical, and hairy pods that contain seeds varying in number from 7 to 20. These seeds, small in size, are ellipsoid or cube-shaped and display a range of colors. While typically green, they can also appear in hues of yellow, olive, brown, purplish brown, or black [5].

Over past three decades, productivity of pulse crops has seen limited growth due to challenges in developing improved varieties, exacerbated by their cultivation in marginal and sub-marginal lands. These crops face an array of issues including fungi, bacteria, viruses, nematodes, and abiotic stressors all cause illnesses [6]. Among these challenges, green gram, in particular, contends with various fungal diseases such as powdery mildew (*Erysiphe polygoni* DC), anthracnose (*Colletotrichum lindemuthianum* Sacc. & Magnus), blight (*Thanatephorus cucumeris* (Frank) Donk), dry root rot (*Macrophomina phaseolina* (Tassi) Goid), leaf spot (*Cercospora canescens* Ellis & G. Martin), and rust (*Uromyces phaseoli* (Persoon) G. Winter). Additionally, viral infections like Mung bean yellow mosaic virus and Leaf crinkle virus pose threats to green gram, while bacterial blight is also observed in this crop. Among these diseases, anthracnose significantly impacts the yield of green gram [7]. Anthracnose causes severe leaf spotting, leading to 'shot hole' symptoms and eventual defoliation, greatly affecting yield. Pod infections directly harm seeds, reducing their germinability. Studies indicate that anthracnose caused a 40.18% average seed yield loss and a 46.90% stalk yield loss in green gram [8]. Anthracnose in green gram is more common during the kharif season in south Gujarat due to predominant favourable weather conditions throughout the crop season [9].

Given the conducive weather conditions during the kharif season in South Gujarat, anthracnose in green gram crops becomes more prevalent. Hence, conducting a study specifically focusing on the anthracnose in green gram becomes crucial. This study highlights the necessity for biocontrol agents to effectively manage this disease and emphasizes the screening of various seasonal varieties against this pathogen having importance in breeding programme.

2. MATERIALS AND METHODS

2.1 *In vitro* Evaluation of Biocontrol Agents

Five biocontrol agents were assessed using the dual culture method. *Trichoderma virens, Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens,* and *Bacillus subtilis* were employed as antagonists. The procedure involved utilizing seven-day-old cultures of the pathogen (*Colletotrichum lindemuthianum*) and the bioagents. Maintaining a distance of 60 mm between the antagonist and the test pathogen, a 5 mm diameter mycelial disc was placed at the Petri plate's edge. For bacterial bioagents, streaking was performed. The control setup contained only the test pathogen positioned at the center of the Petri plate. These Petri plates underwent a 7-day incubation period in a BOD incubator at a constant temperature of 27°C. Observations on mycelial development and the percentage of growth inhibition (PGI) were recorded after this incubation period. The PGI for each treatment was calculated using the formula [10] (see Table 1).

$$PGI = \frac{C - T}{C} \times 100$$

Where,

PGI = percentage of growth inhibition
C=Colony diameter (mm) in control plate
T=Colony diameter (mm) in treated plate.

2.2 Screening of Varieties Against Anthracnose Disease of Green Gram

In a controlled greenhouse environment, eight genotypes were planted in earthen pots, maintaining a temperature of 25°C and a relative humidity of 90%. The pots were filled with a mixture of sterilized farmyard manure and soil in a 1:3 ratio. A conidial suspension of *C. lindemuthianum*, comprising 2×10^5 conidia/ml, was prepared from a ten-day-old culture. Seedlings were infected with this suspension 20 days after sowing. The percent disease intensity (PDI) was determined using the disease severity scale (see Table 2) specified for anthracnose of green gram [11]. Table 3 presents a list of varieties categorized by season.

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaf observed x maximum rating}} \times 100$$

Table 1. *In vitro* evaluation of different antagonist against *Colletotrichum lindemuthianum*

Treatment	Name of bio agent
T_1	*Trichoderma harzianum*, NAU isolate
T_2	*Trichoderma viride*, NAU isolate
T_3	*Trichoderma virens*, NAU isolate
T_4	*Bacillus subtilis*, NAU isolate
T_5	*Pseudomonas fluorescence*, NAU isolate
T_6	Control

Table 2. Disease severity scale for anthracnose of green gram (Mayee and Datar,1986)

Severity scale	Description
0	No infection
1	Small size lesions covering 1% or less of leaf area
3	Small size lesions covering 1-10% of leaf area
5	Lesions size big but not coalescing, covering 11-25% of the leaf area
7	Lesions on leaves covering 26-50% of leaf area. Cankers on stem and pod infection
9	Lesions on leaves covering 51% or more of leaf area. Defoliation of leaves, deep cankers on stem and pods, blighting of plant occurs

Table 3. Varieties used in screening of anthracnose disease

Kharif varieties	Rabi varieties	Summer and kharif varieties
KM 2328	TARM-18	Pusa 1431
Vaibhav	GBM-1	GM-6
BPMR-145	CO-4	-

2.3 Statistical Analysis

Before conducting ANOVA, normality and homogeneity of variance tests were conducted using the data from both the dual cultures and percent disease intensity. Square root transformation was applied to the dual culture data, while arc sine transformation was employed for the percent disease intensity data. All analyses considered P-values below 0.05 as indicative of statistical significance.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of Biocontrol Agents

The *in vitro* study revealed significant inhibition of the test pathogen's growth using indigenous antagonist isolates *via* the dual culture method. Table 4 presents data on the average colony diameter of the pathogen and the corresponding percent inhibition, while

Fig. 1. and Graph 1 visually represent these results. Overall, all antagonists except *P. fluorescens* demonstrated substantial effectiveness in restraining the growth of *C. lindemuthianum*. With the exception of *P. fluorescens*, all other antagonists exhibited over 30% inhibition of the test fungus. Notably, *T. virens* (3.41 mm) and *T. viride* (3.69 mm) showcased significantly reduced 751 ycelia growth of the pathogen, with *T. virens* exhibiting the maximum inhibition (87.63%). Following closely in effectiveness were *T. harzianum* (4.00 mm) and *B. subtilis* (5.70 mm), while *P. fluorescens* (7.36 mm) displayed comparatively higher 751ycelia growth.

In terms of percent growth inhibition, *T. virens* emerged as the most superior among all tested antagonists, achieving a remarkable 87.63% inhibition. *T. viride* (85.41%), *T. harzianum* (82.78%), and *B. subtilis* (64.44%) also displayed considerable effectiveness against the pathogen. However, *P. fluorescens*, while showing some efficacy, exhibited a relatively moderate inhibition at 40.41% compared to the other antagonists.

Similar findings were reported in various studies assessing the efficacy of screened biocontrol agents against different pathogens. It demonstrated that biocontrols against *C. dematium* and *T. koingii* exhibited maximum growth inhibition, closely followed by *T. harzianum* [12]. *Trichoderma viride* at a concentration of 0.4% displayed a 50% and 52% disease index for leaf anthracnose and pod blight, respectively. Additionally, the superiority of *T. harzianum* in suppressing *C. trancatum* growth over *T. viride* was noted in one study [13]. In terms of bacterial biocontrol agents, *Bacillus subtilis* (TNAU) isolates showed maximum suppression of 751ycelia growth [14]. In another investigation against *C. gloeosporiodes* under *in vitro* conditions, *T. harzianum* exhibited the highest inhibition, closely followed by *Pseudomonas fluorescens* after 5 days of incubation [15]. *T. harzianum* caused 100% inhibition for all the *C. truncatum* causing anthracnose of green gram. *T. viride* inhibited the fungal growth by 69.44%. *B. subtilis* inhibited 55.74% and least inhibition of fungal 751ycelia growth was noticed by *P. fluorescens* [16]. Among the fungal bioagents screened against *C. truncatum,* the highest 751 ycelia inhibition was found in the *T. viride*. While among the bacterial bioagents, *B. subtilis* and *P. fluorescens* showed highest 751ycelia inhibition [17].

3.2 Screening of Green Gram Varieties against Anthracnose Disease

In pursuit of identifying resistance against green gram anthracnose, eight different green gram germplasms were screened under controlled greenhouse conditions at the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India during 2019-20. The standard procedure was adhered to throughout the assessment. Observations on PDI were meticulously recorded.Utilizing the 0-9 disease rating scale established by Mayee and Datar (1986), disease scores were assigned before flowering, during pod formation, and at physiological maturity. PDI derived from these scores is presented in Table 6 and depicted in Fig. 2. Table 5 categorizes the disease reaction based on PDI values.None of the genotypes exhibited immunity against the

pathogen. However, Pusa 1431 (5.49%), KM-2328 (6.12%), TARM-18 (7.18%), and GM-6 (16.22%) displayed a resistant reaction to anthracnose. GBM-1 (23.56%) and CO-4 (26.58%) demonstrated moderate resistance. No variety was classified as highly susceptible, yet Vaibhav (56.25%) and BPMR-145 (58.16%) exhibited susceptibility to green gram anthracnose.

Table 4. *In vitro* evaluation of different bioagents against *Colletotrichum lindemuthianum*

Sl. No.	Bioagents	Average diameter of pathogen (mm)@	Growth inhibition (%)
1	*Trichoderma harzianum* NAU isolate	4.00* (15.50)**	82.78
2	*Trichoderma viride* NAU isolate	3.69 (13.13)	85.41
3	*Trichoderma virens* NAU isolate	3.41 (11.13)	87.63
4	*Bacillus subtilis* NAU isolate	5.70 (32.00)	64.44
5	*Pseudomonas fluorescens* NAU isolate	7.36 (53.63)	40.41
6	Control	9.51 (90.00)	-
S.Em. ±		0.06	
C.D. at 5%		0.19	
C.V. %		2.24	

@ Average of four repetitions
** Figures outside parenthesis are √x+0.5 transformed value*
*** Figures in parenthesis are original values*

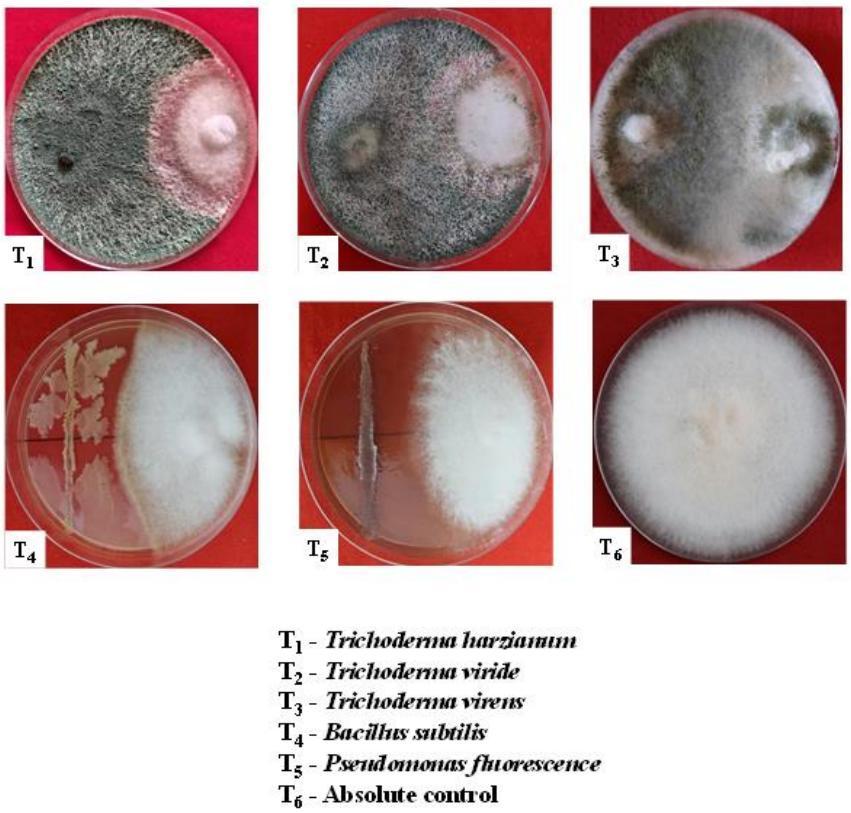
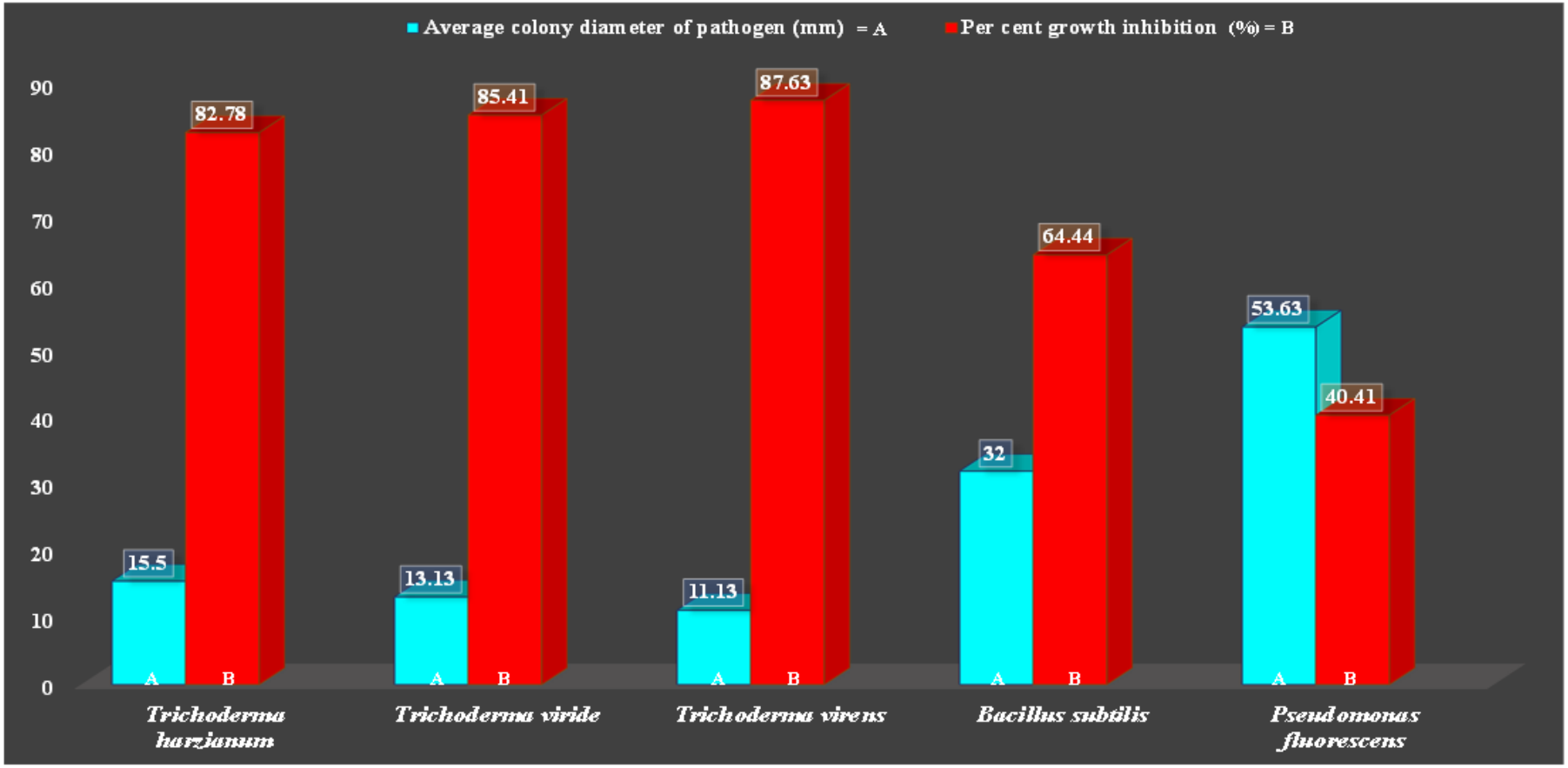


Fig. 1. *In vitro* growth inhibition of *C. lindemuthianum* on PDA with different biocontrol agents



Graph 1. Grapical representation of average colony diameter of pathogen and per cent growth inhibition of bioagents

Table 5. Categorisation of disease reaction on the basis of PDI value.

Sl. No.	PDI range	Disease reaction
1.	0%	Immune
2.	0 to 20%	Resistant
3.	21 to 35%	Moderately resistant
4.	36 to 45%	Moderately susceptible
5.	46 to 70%	Susceptible
6.	More than 70%	Highly susceptible

Table 6. Reactions of green gram varieties against *Colletotrichum lindemuthianum* in pot culture

Variety/ Germplasm	PDI %	Disease reaction
Pusa 1431	*13.61 ** (5.49)	Resistant
GM-6	23.81 (16.22)	Resistant
Vaibhav	48.65 (56.25)	Susceptible
BPMR-145	49.75 (58.16)	Susceptible
CO-4	31.09 (26.58)	Moderately Resistant
KM-2328	14.38 (6.12)	Resistant
TARM-18	15.60 (7.18)	Resistant
GBM-1	29.09 (23.56)	Moderately Resistant
S.Em.± C.D. at 5% C.V. (%)		0.02 0.06 0.15

@ Average of three repetitions
**Figures outside parenthesis are arc sine transformed value*
***Figures in parenthesis are original value*

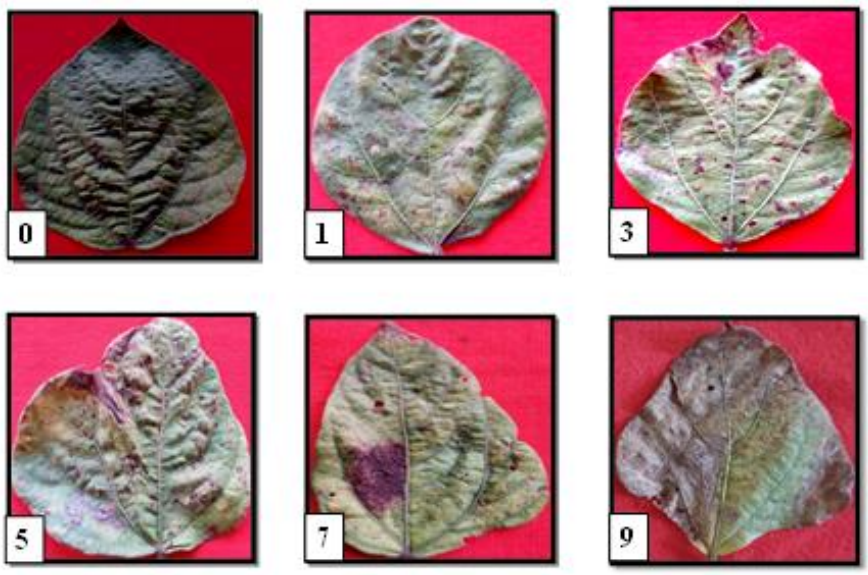
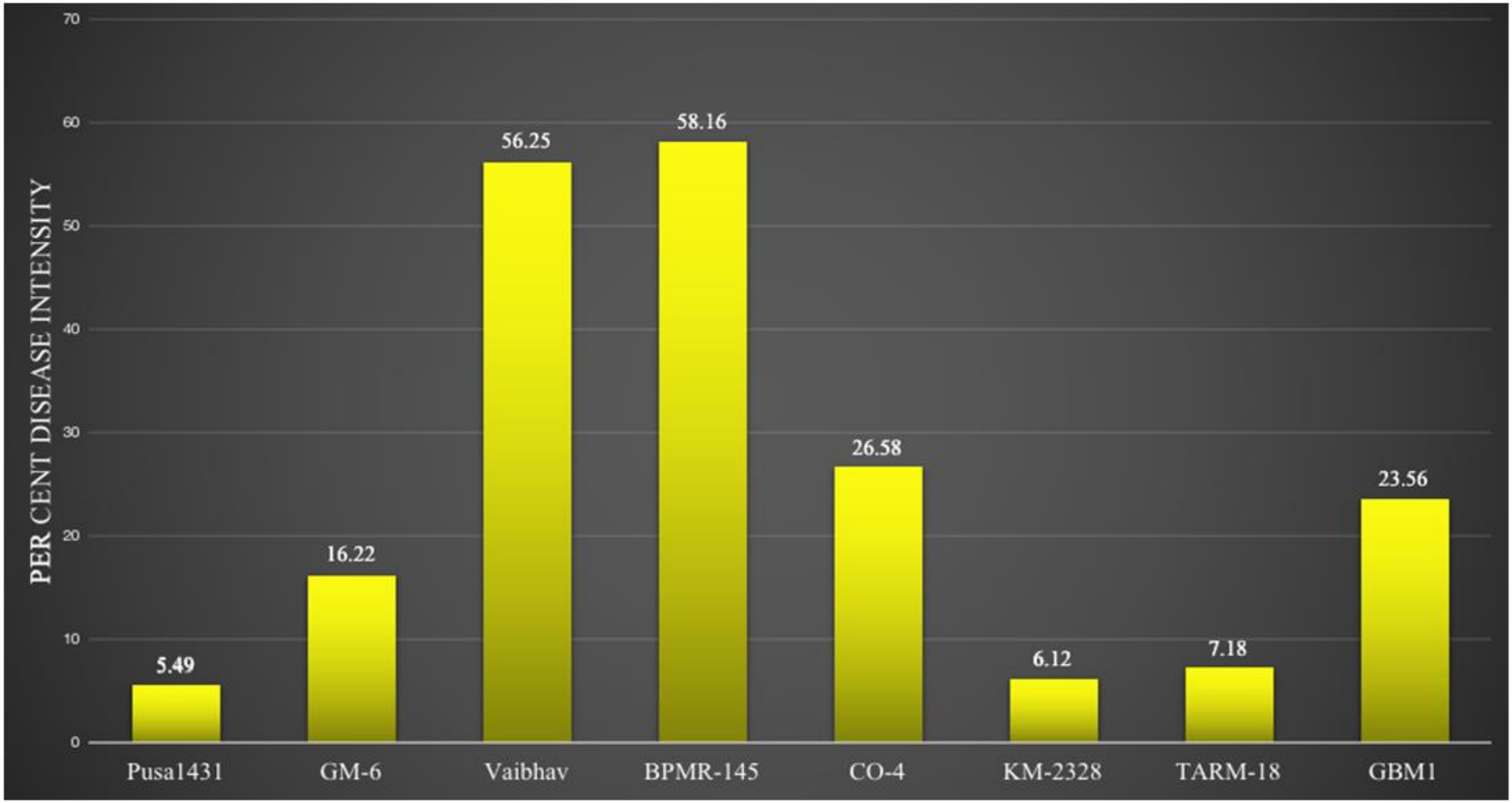


Fig. 2. Disease rating sacle of anthracnose of green gram given by Mayee and Datar (1986)



Graph 2. Graphical representation of per cent disease intensity of varieties against *C. lindemuthianu*

Previous studies conducted by researchers have reported similar results regarding green gram anthracnose. Several genotypes, including TM-96-2 and TARM-18, showed a resistant reaction, whereas BGS-9, TM-98-50, and TM-97-55 showed moderately resistant reactions. Other genotypes, on the other hand, others have been categorised as susceptible to highly susceptible in their reaction to the disease [8]. In a field screening involving sixty-five mungbean genotypes conducted under natural epiphytotic conditions, identified two genotypes (LGG-460 and TMV-37) were found to be resistant, one (GM-9926) to be somewhat resistant, and twenty-five genotypes to be moderately sensitive to anthracnose. The remaining genotypes were discovered to be extremely susceptible to susceptible [18]. Under *in vivo* conditions, observed that out of 38 genotypes, with the exception of Sonali, PM-4, and Pusa-1174, which showed somewhat sensitive reactions, the majority were generally resistant. Nonetheless, certain genotypes showed resistance to the disease, including Sukumar, PM-D5, TARM-18, and CZMK-1 [19]. In another assessment cultivars PU-31 and PU-30 demonstrated moderate resistance, while PU-38 and PU-40 showed moderate susceptibility. TAU-1 and PUI-94-1 were classified as susceptible cultivars [20].

4. CONCLUSION

The experiment highlighted the effectiveness of *T. virens* and *T. viride* in controlling anthracnose disease in green gram. This effectiveness stemmed from the competition between these bioagents and the pathogen for nutrients and space. Specific green gram varieties like Pusa 1431, KM-2328, GM 6, and TARM-18 demonstrated a resistant reaction against green gram anthracnose. Utilizing biocontrol agents is a cost-effective approach in disease management. Furthermore, it's a safe method that doesn't induce toxicity in crop plants. Application of biocontrol agents proves safer for the environment and the individuals applying them. Integrating resistant varieties stands out as a solid strategy in disease management. The use of resistant and high-yielding varieties emerges as an environmentally safe, economically viable approach. It's a less expensive technique for disease management and proves financially beneficial for farmers. Although immune genotypes weren't found in the current study, the presence of resistant genotypes plays a pivotal role in integrated disease management. Moderately resistant varieties effectively limit field spread and reduce conidia production, contributing significantly to disease control strategies. Recognizing these sources of resistance remains a crucial aspect in breeding for disease-resistant varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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