



RESEARCH ARTICLE

Insights into chilli anthracnose management: Unraveling the population dynamics of *Colletotrichum truncatum*

Desetty J M S N K Sri Veda¹, Pravallika Sree Rayanoothala^{2*} & Prabir Chakraborti³

¹Department of Genetics and Plant Breeding & Seed Science and Technology, M S Swaminathan school of agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha 761 211, India

²Department of Plant Pathology, M S Swaminathan school of agriculture, Centurion University of Technology and Management, Paralakhemundi, Odisha 761 211, India

³Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia 741 252, India

*Correspondence email - rpravallikasree@gmail.com

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Abstract

Chilli (*Capsicum annum* L.), commonly known as red pepper, is a vital spice crop in India. However, its productivity has been adversely affected in recent years due to various biotic and abiotic stresses. While fungicides have been effective in mitigating these diseases, their continued use poses significant environmental and health risks and contributes to the emergence of fungicide-resistant pathogens. Consequently, there is a growing need to adopt sustainable and eco-friendly disease management strategies, such as the use of bio-inoculants. Bio-priming, an innovative seed treatment technique that combines hydration and biological inoculation has been successfully employed in many vegetable crops but remains underexplored in chilli, particularly for disease control. In this study, chilli seeds were bio-primed with either *Trichoderma harzianum* or *Pseudomonas fluorescens* or their combination. The results demonstrated that the combined application of *T. harzianum* and *P. fluorescens* significantly reduced the Percent Disease Index (PDI) compared to untreated seeds. *T. harzianum* applied alone also showed considerable effectiveness in disease management. Anthracnose incidence was assessed using PDI values at 35 and 60 days after transplanting (DAT) during both the pre-kharif and rabi seasons. A higher disease incidence was observed in the rabi season. Among the genotypes tested, 'Anugraha' exhibited superior resistance to anthracnose post-bio-priming, consistently recording lower PDI values under all observations. In contrast, the variety, 'Suryamukhi Bullet', was more susceptible, with significantly higher PDI values.

Keywords: anthracnose; bio-priming; chilli; *Pseudomonas*; *Trichoderma*

Introduction

Chilli or red pepper, (*Capsicum annum* L.), is a prominent fruit (Family: *Solanaceae*, solitary or cymose type of inflorescence) that is used as a spice in India and other countries. Chilli peppers are used in cooking and are also high in fiber, protein, vitamins A, C and E, capsaicin, capsochrome, potassium and folic acid (1). In particular, capsaicin has anti-inflammatory, anti-cancer, antibacterial, antioxidant and immunomodulatory qualities that are beneficial to health. With over 80% of the country's cultivation area coming from major chilli-growing states including Orissa, West Bengal, Andhra Pradesh, Maharashtra, Karnataka, Rajasthan, Uttar Pradesh and Tamil Nadu, India is the world's largest producer, user and exporter of dried peppers (2). Using 0.797 million ha of land for cultivation of chilli, India produces roughly 0.0679 million tons of green chilli and 1.389 million tons of dried chilli, according to recent data (3, 4). However, several biotic and abiotic stress factors have recently put chilli yields in jeopardy. These losses are largely caused by fungi, including anthracnose, Fusarium wilt, damping-off, collar rot and stem rot. A notable consequence of anthracnose (*Colletotrichum*

truncatum) has been a 50% reduction in worldwide yield (5, 6). Plant growth-promoting fungi (PGPF) and bacteria (PGPB) are effective biocontrol agents because they promote plant growth and development by producing hormones and siderophores, colonizing roots and enhancing nutrient uptake mechanisms (7). These agents provide long-lasting protection against pathogens through a variety of mechanisms, including antibiosis, production of secondary metabolites, degradation of cell walls by enzymes and deposition of callose and lignin in the cell wall (8). They also lessen the severity of disease and help plants develop systemic resistance (3).

Trichoderma spp. are soil-dwelling organisms that inhibit the growth of soil-borne pathogens through diverse mechanisms like competition for nutrients and space and induction of systemic resistance in plants (9, 10). Given the urgent need for novel strategies to manage anthracnose disease, leveraging the host plant's natural immunity to develop systemic resistance is promising (11). This study aims to validate the efficacy of bioinoculants *T. harzianum* and *P. fluorescens* used in seed priming under field conditions (12).

The economic implications of anthracnose disease in chilli are substantial, affecting not only the livelihood of smallholder farmers but also the overall agricultural export revenue of chilli-producing nations like India. Anthracnose leads to significant post-harvest losses by damaging the fruit quality and marketability. The pathogen can remain latent and resurface under favorable environmental conditions, making its management more complex. Moreover, changing climatic conditions, including increased humidity and temperatures, have further exacerbated the disease prevalence, necessitating innovative and sustainable management approaches. Conventional methods of managing anthracnose, including chemical fungicides, are becoming less effective due to the emergence of resistant pathogen strains. Additionally, these chemicals pose environmental risks and are often costly, limiting their accessibility to resource-constrained farmers. The overuse of chemical fungicides has raised concerns about food safety, soil health and ecological balance. This has led to a paradigm shift toward integrating biological control agents into disease management programs, offering an environmentally friendly and cost-effective alternative.

Recent advancements in molecular biology and biotechnology have opened new avenues for improving disease resistance in crops. By understanding the molecular interactions between *C. truncatum* and chilli plants, researchers can identify key resistance genes and mechanisms. The integration of bioinoculants, such as *T. harzianum* and *P. fluorescens*, with resistant chilli varieties offers a synergistic approach to combating anthracnose. Furthermore, field-based validation studies like the present one are critical for ensuring that these solutions are not only effective, but also practical for adoption in diverse agro-climatic conditions.

Biopriming is an innovative and eco-friendly seed treatment technology that integrates biological agents, hydration processes and priming techniques to enhance plant health and performance. This method involves treating seeds with beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR), fungi or other biological inoculants to improve seed germination, vigor and resistance against biotic and abiotic stresses. Biopriming not only enhances early seedling establishment but also induces systemic resistance in plants through the activation of defense mechanisms. Studies have demonstrated its efficacy in improving resistance to various pathogens by modulating the expression of defense-related genes, making it a sustainable approach for modern agriculture (12). Furthermore, it plays a vital role in reducing dependency on chemical pesticides and fertilizers, aligning with global sustainability goals.

Recent advancements in biopriming include the integration of molecular tools to identify novel microbial strains and optimize their interactions with host plants, thus opening new avenues for precision agriculture (13). Its application in rice, particularly against bacterial blight, holds significant promise for enhancing crop resilience and productivity in resource-limited systems.

Materials and Methods

Data collection

An *in vivo* experiment was conducted in 2021 and 2022 at the Bidhan Chandra Krishiviswavidyalaya, Mandouri Farm, Mohanpur, Nadia, West Bengal, India (Latitude: 22.9402° N, Longitude: 88.5328° E.) The strains of *P. fluorescens* and *T. harzianum* (Table 1) were obtained from pathology lab in the form of talc formulations and used as four treatments and were replicated thrice. From the Department of Plant Pathology, B.C.K.V., Mohanpur, *Pseudomonas* spp. and *Trichoderma* spp. containing 10⁸ cfu/g were obtained for biopriming (13).

Genotype details

The study used the following varieties of chilli: Anugraha (V1), G4 (V2), LCA-625 (V3), Masinga Morok (V4) and Suryamukhi Bullet (V5). After sterilizing the seeds for 2-5 min with 0.1% HgCl₂, they were rinsed twice or thrice with distilled water. Following sterilization, the seeds underwent a designated period of sowing in water and subsequently, 10 g/kg seed was combined with bioinoculants. For 12 hr, the mixture of seeds and bioinoculants was heaped, covered with a moist jute sack to retain high humidity and incubated in a shaded area. Following the incubation period, the seeds were placed in nursery beds, where the seed coat had developed a protective layer due to the bio agents (13).

Percent Disease Index (PDI) of anthracnose

To assess the PDI of anthracnose, 10 plants per plot were selected for observation. Three trifoliate leaves from the base, middle and upper portions of each plant were examined for symptoms of anthracnose (sunken necrotic tissue through concentric rings of acervuli (Fig. 1)). Observations were recorded at 35 and 60 days after sowing (DAS) during the pre-kharif stage using a standard graded scale (0-5) for anthracnose disease (14, 15) (Table 2).

PDI =

$$\frac{\sum \text{of ratings of infected leaves observed}}{\text{No. of leaves observed} \times \text{Maximum}} \times 100 \text{ disease score}$$

(Eqn. 1)

Table 1. Treatment details

Treatments	Bio-priming
T1	<i>T. harzianum</i> for 12 h
T2	<i>P. fluorescens</i> for 12 h
T3	<i>T. harzianum</i> + <i>P. fluorescens</i> for 12 h
T4 (Control)	Hydropriming for 12 h

Table 2. Disease scoring scale (0–5) for anthracnose disease

Disease scale	Percent infection	Category
0	No visible symptom	Immune
1	0.1–5.0%	Highly resistant
2	5.1–10.0%	Resistant
3	10.1–50.0%	Moderately resistant
4	51.1–90.0%	Susceptible
5	> 90.0%	Highly susceptible



Fig. 1. Photograph showing the characteristic symptoms of *Colletotrichum truncatum*.

Results

Effect of bio-inoculants on anthracnose disease at different growth stages

Table 3 through 6 illustrate the effectiveness of bio-inoculants in reducing anthracnose disease incidence across chilli growth stages during the pre-kharif and rabi seasons. The PDI of anthracnose leaf spot was recorded at 35 DAS and 60 DAS across five varieties of chilli during both seasons. Significant variations were observed among varieties, seasons and plant growth stages.

The PDI of bio-primed chilli varieties showed a substantial reduction compared to the control. This reduction was most evident in treatments with bio-inoculants during both 35 DAS and 60 DAS, highlighting the effectiveness of seed priming treatments. Variety V1 demonstrated the lowest PDI, followed by V2, while the maximum PDI was consistently observed in V4 at both growth stages and seasons.

Variation in AUDPC values across treatments

The Area Under Disease Progress Curve (AUDPC) values revealed significant differences among treatments. T2 (*T. harzianum*) exhibited the lowest AUDPC value (5.00), indicating superior disease control, while T4 (Control) recorded the highest AUDPC value (9.61), suggesting maximum disease progression. Fig. 2 through 5 depict these variations, clearly demonstrating that bio-priming significantly curbs disease progression.

Disease resistance mechanisms and genotypic responses

The efficacy of bio-inoculants like *T. harzianum* and *P. fluorescens* lies in their ability to enhance plant systemic

resistance. Priming with these bioagents strengthens cell walls through the deposition of cellulose and lignin and induces protective enzyme synthesis. For instance, *P. fluorescens* BAM-4 produces antifungal metabolites like siderophores and chitinase, which reduce pathogen invasion while enhancing antioxidative enzyme activities and phenolic compound synthesis. This study highlights the cumulative disease suppression effect using AUDPC, underscoring its advantage over single-point severity assessments. Lower AUDPC values for bio-primed genotypes, particularly in T2, indicate reduced disease progression and improved resistance against anthracnose.

Discussion

The findings affirm that bio-priming with *T. harzianum* and *P. fluorescens* effectively mitigates anthracnose in chilli, supporting previous studies. These bioagents not only suppress pathogen development but also bolster plant defense mechanisms through systemic resistance. The integration of these treatments into crop management practices can lead to enhanced disease control, improved crop health and sustainable agricultural practices.

Although bio-priming is frequently employed in vegetable crops, it is rather uncommon in chilli crops, particularly when it comes to disease control. On the other hand, bio-priming has continuously proven to be successful in eliminating infections and promoting seedling establishment. PGPB and PGPF together improve seedling quality, stability and uniformity while lowering disease incidence (16). Biological control agents (BCAs) generate enzymes and secondary metabolites that prevent pathogens from invading, growing and settling in plants (17). Microorganisms classified as phyllospheric and rhizospheric (PGPB and PGPF) are essential for the growth and development of vegetation as well as for mitigating pathogen incursion through foliar and soil attacks. Mechanical tissues are strengthened by priming with bio-inoculants such as *T. harzianum* and *P. fluorescens*, which deposit cellulose and lignin, build solid cell walls and encourage the production of protecting enzymes, ultimately resulting in development of systemic resistance (18). For instance, *P. fluorescens* BAM-4 generates metabolites such as the iron-based siderophore and the antifungal defense enzyme, chitinase (19). These metabolites reduce pathogen attacks from seeds and soil by causing plants to develop systemic resistance. As seen in chilli, bio-priming with these

Table 3. Effect of bio-inoculants on anthracnose leaf spot of chilli disease incidence at 30 DAT in pre-kharif season

Varieties	Treatments				Mean
	T1	T2	T3	T4	
V1	0.10 (1.72)	0.08 (1.79)	0.09 (3.23)	0.27 (4.04)	0.14
V2	0.12 (1.60)	0.10 (1.84)	0.11 (4.08)	0.29 (3.20)	0.16
V3	0.34 (1.64)	0.35 (3.07)	0.32 (3.68)	0.51 (3.25)	0.38
V4	0.41 (2.96)	0.43 (3.35)	0.40 (3.77)	0.50 (3.07)	0.44
V5	0.31 (1.90)	0.32 (3.40)	0.29 (3.58)	0.48 (3.96)	0.35
Mean	0.26	0.26	0.24	0.41	
Factors	V		T		T×V
SE(m)	0.061		0.055		0.122
CD(p≤0.05)	0.176		0.157		0.351

Table 4. Effect of bio-inoculants on anthracnose leaf spot of chilli disease incidence at 60 DAT in pre-kharif season

Varieties	Treatments				Mean
	T1	T2	T3	T4	
V1	0.20 (2.70)	0.18 (2.82)	0.19 (3.47)	0.38 (4.47)	0.24
V2	0.22 (2.82)	0.24 (2.60)	0.21 (4.24)	0.40 (3.35)	
V3	0.38 (2.60)	0.40 (3.61)	0.37 (3.77)	0.55 (3.45)	0.43
V4	0.44 (3.61)	0.45 (3.49)	0.42 (3.85)	0.61 (3.28)	
V5	0.34 (2.70)	0.36 (3.59)	0.33 (3.70)	0.52 (4.12)	0.39
Mean	0.32	0.33	0.30	0.49	
Factors	V				T×V
SE(m)	0.023				0.045
CD(p≤0.05)	0.065				0.131

SE: Standard error; DAT: Days after transplanting; CD: Critical difference

Table 5. Effect of bio-inoculants on anthracnose leaf spot of chilli disease incidence at 30 DAT in rabi season

Varieties	Treatments				Mean
	T1	T2	T3	T4	
V1	0.22 (2.63)	0.20 (2.68)	0.20 (3.79)	0.39 (4.68)	0.25
V2	0.24 (2.55)	0.22 (2.73)	0.23 (4.54)	0.41 (3.72)	
V3	0.46 (2.54)	0.47 (3.66)	0.44 (4.39)	0.63 (3.77)	0.50
V4	0.59 (3.57)	0.54 (3.90)	0.48 (4.19)	0.67 (3.66)	
V5	0.43 (2.76)	0.44 (3.94)	0.41 (3.95)	0.59 (4.40)	0.47
Mean	0.39	0.37	0.35	0.54	
Factors	V				T×V
SE(m)	0.040				0.081
CD(p≤0.05)	0.116				0.232

SE: Standard error; DAT: Days after transplanting; CD: Critical difference

Table 6. Effect of Bio-inoculants on Anthracnose leaf spot of chilli disease incidence at 60 DAT in rabi season

Varieties	Treatments				Mean
	T1	T2	T3	T4	
V1	0.27 (2.99)	0.26 (3.02)	0.26 (4.00)	0.45 (4.77)	0.31
V2	0.29 (2.89)	0.28 (3.02)	0.28 (4.72)	0.47 (3.98)	
V3	0.52 (2.90)	0.52 (3.92)	0.49 (4.49)	0.68 (4.02)	0.55
V4	0.62 (3.83)	0.56 (4.10)	0.51 (4.30)	0.69 (3.88)	
V5	0.48 (3.09)	0.49 (4.14)	0.46 (4.07)	0.65 (4.61)	0.52
Mean	0.43	0.42	0.40	0.59	
Factors	V				T×V
SE(m)	0.038				0.075
CD(p≤0.05)	0.108				0.216

SE: Standard error; DAT: Days after transplanting; CD: Critical difference

substances increases defense, antioxidative enzymes and phenolic compound synthesis while decreasing lesion progression and ROS formation (20). AUDPC of different chilli genotypes, when plotted treatment wise, varied greatly between treatments (Fig. 2-5). In the present study, for anthracnose, the AUDPC values were found to be minimum in T2 (*T. harzianum*) (5.00) and maximum in T4 (control) (9.61).

The response of resistant chilli genotypes to the aforementioned is influenced by a number of other parameters, such as the existence or absence of pathotypes and races and all these combine to have a big impact on how this disease spreads. The advantage of using AUDPC scores

over a single severity test is that they indicate disease progression over the whole growing period (21). A more accurate evaluation of phenotypic characteristics is produced by AUDPC's use of many evaluations that are independent of transformations (22). The genotype/treatment is more vulnerable if there is higher AUDPC. This study examined the disease's cumulative progression using AUDPC, which differed significantly among the various treatments, indicating low cumulative disease progress in T2 (*T. harzianum*) when used as bioinoculant (23).

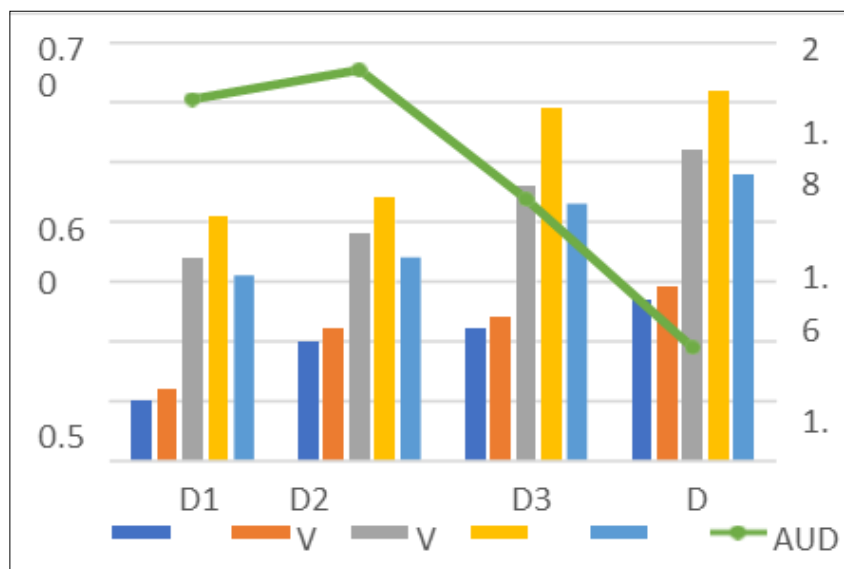


Fig. 2. Relation between DI & AUDPC for different chilli genotypes screened after bio-priming under field conditions for anthracnose disease with T1 (*P. fluorescens*).

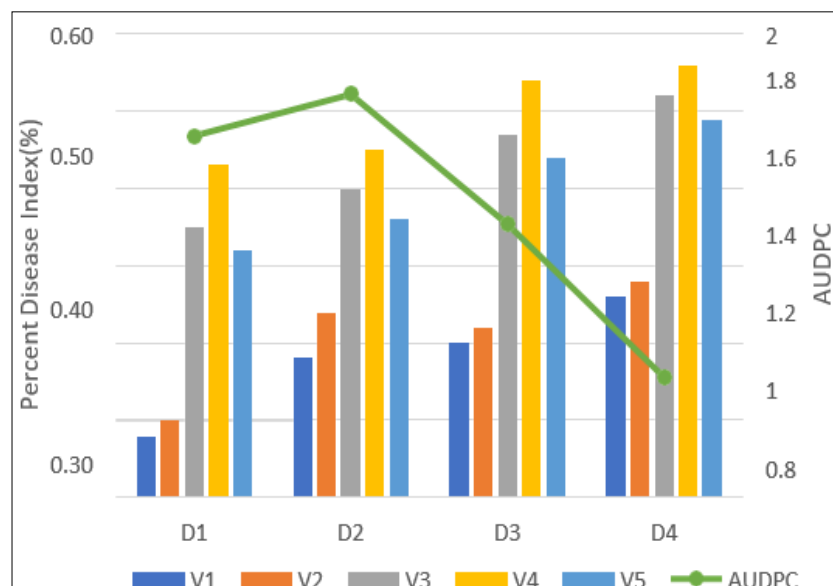


Fig. 3. Relation between DI & AUDPC for different chilli genotypes screened after bio-priming under field conditions for anthracnose disease with T2 (*T. harzianum*).

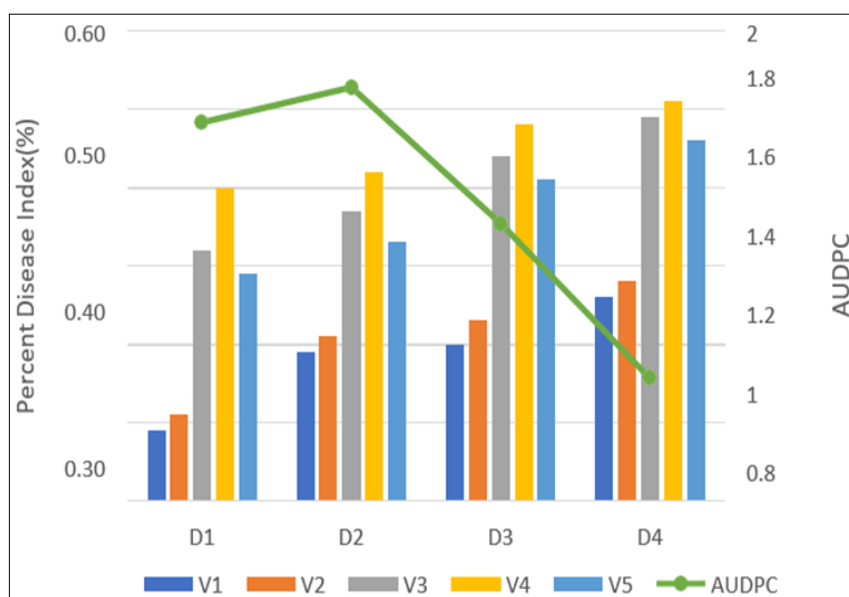


Fig. 4. Relation between DI & AUDPC for different chilli genotypes screened after bio-priming under field conditions for anthracnose disease with T3 (*T. harzianum* and *P. fluorescens*).

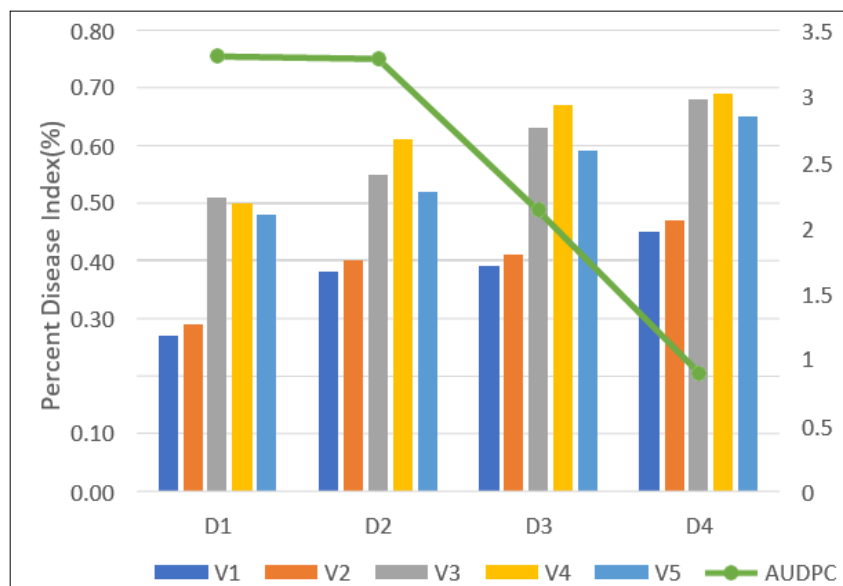


Fig. 5. Relation between DI & AUDPC for different chilli genotypes screened after bio-priming under field conditions for anthracnose disease with T4 (Control).

Conclusion

The PDI values during the pre-kharif and rabi seasons were analyzed to determine the incidence of anthracnose, with the rabi season showing a higher incidence compared to the pre-kharif season. In disease suppression, the T3-treated plants performed significantly better than T1 and T2 in both the pre-kharif and rabi seasons. The variety V1 exhibited superior resistance post-biopriming, indicated by a reduced PDI in all observations. Conversely, the bio-primed variety V4 showed a higher incidence of disease, indicating its susceptibility. However, variation among treated plants was observed at both stages and across both seasons compared to the control. These agents are profitably used in the agricultural industry as environment friendly biofertilizers and biopesticides. The current experiment successfully produced chilli fruit and seeds, highlighting the importance of genotype selection and cultivation season.

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Authors' contributions

DSV and PR carried out the research and compiled the manuscript. DSV, PR and PC analyzed and interpreted the data. PC read and reviewed the manuscript, conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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