



# Integrated Management of White Rust (*Albugo candida*) in Indian Mustard Using Biocontrol Agents under Field Conditions

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

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## Abstract

White rust of Indian mustard (*Brassica juncea* L.), caused by the obligate oomycete *Albugo candida* (Pers.) Kuntze, represents one of the most economically devastating biotic constraints to mustard cultivation across diverse agro-climatic regions of India. The disease is particularly severe during the Rabi (winter) cropping season, when cool temperatures and high relative humidity create ideal conditions for pathogen proliferation and disease development. The present field investigation was systematically conducted during the Rabi season of 2024-25 at the Agricultural Research Farm of Vikrant University, Gwalior, Madhya Pradesh, with the twin objectives of evaluating the efficacy of selected biocontrol agents under field conditions and analyzing disease severity trends across major mustard-growing agro-climatic zones of India.

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The experiment was designed following a randomized block design (RBD) with four replications and seven distinct treatment combinations comprising *Trichoderma viride*, *Pseudomonas fluorescens*, their synergistic combination, botanical resistance inducers (garlic extract and salicylic acid), a fungicide standard (Mancozeb 75% WP), and an untreated control. Critical parameters including disease incidence (%), percent disease index (PDI), and seed yield ( $\text{kg ha}^{-1}$ ) were recorded at weekly intervals across the entire crop growth period from November 2024 to February 2025.

Experimental results demonstrated that the combined application of *Trichoderma viride* and *Pseudomonas fluorescens* was significantly superior among all biocontrol treatments, reducing disease incidence to 22.1% and PDI to 26.7%, representing a 52.9% disease reduction over the untreated control. This treatment also produced the highest seed yield among biocontrol options at  $1580 \text{ kg ha}^{-1}$ , reflecting a 54.9% yield advantage over the control ( $1020 \text{ kg ha}^{-1}$ ). The chemical fungicide Mancozeb 75% WP outperformed all other treatments with a PDI of 23.4% and maximum yield of  $1650 \text{ kg ha}^{-1}$ , serving as an effective benchmark. Regional analysis confirmed higher disease pressure in eastern and north-eastern India due to elevated humidity and temperature fluctuations. The study strongly advocates for the integration of biocontrol agents into sustainable, eco-friendly integrated disease management (IDM) programmes for mustard production systems in India.

**Keywords:** Mustard; white rust; *Albugo candida*; biocontrol agents; *Trichoderma viride*; *Pseudomonas fluorescens*; integrated disease management; *Brassica juncea*.

## 1. Introduction

### 1.1 Importance of Mustard as an Oilseed Crop

Indian mustard (*Brassica juncea* L. Czern. & Coss.) holds a pivotal position in the agricultural economy of India, ranking as the second most important oilseed crop after groundnut in terms of cultivated area and production (Shekhawat et al., 2012). It serves as the primary source of edible oil for an estimated 50% of the population residing in the major mustard-growing states of Uttar Pradesh, Punjab, Rajasthan, Bihar, Odisha, West Bengal, Madhya Pradesh, and Assam. Beyond its nutritional significance, mustard seeds contain 30–48% oil and are extensively utilized for a broad spectrum of applications ranging from culinary uses and traditional medicine to industrial applications such as biodiesel generation and commercial lubricants (Sachan et al., 2024). Historically, mustard cultivation in the Indian subcontinent dates back to approximately 3000 BC, with Sanskrit texts confirming its early documented use as a spice and medicinal crop (Sharma et al., 2024). During 2014, India's total mustard production stood at approximately 7.32 million tonnes over an area of 6.21 million hectares, recording a productivity of  $1180 \text{ kg ha}^{-1}$ . By the 2021–22 agricultural year, the total oilseed cultivation area in India expanded to approximately 26.4 million hectares, yielding around 36.7 million tonnes of oilseeds collectively. Despite these impressive production statistics, mustard productivity remains substantially below its potential, primarily due to persistent biotic stresses, of which fungal and oomycete diseases remain the most critical (John et al., 2026).

### 1.2 White Rust: A Major Biotic Constraint

White rust, caused by the obligate oomycete pathogen *Albugo candida* (Pers.) Kuntze, is recognized as one of the most destructive diseases affecting the family Brassicaceae, particularly *Brassica juncea* in India. The disease manifests as characteristic chalky white pustules (sori) on the undersurface of leaves, stems, and floral organs. Systemic infection results in distorted floral parts, a condition popularly known as 'staghead' or 'white rust staghead' a particularly damaging phenomenon that directly affects reproductive structures and leads to drastically impaired seed set (Dev et al., 2020).

Under conducive environmental conditions specifically temperature ranges of  $10\text{--}18^\circ\text{C}$ , relative humidity above 85%, and the presence of free moisture on plant surfaces the disease can cause yield losses ranging from 17–34%, and in extreme epidemic years, losses may approach 70–80% in highly susceptible (Priya et al., 2024). The pathogen overwinters as oospores in soil and plant debris and can spread rapidly via airborne sporangia during the crop growth period, making early management interventions critically important (Lavanya, 2025).

### 1.3 Current Management Strategies and their Limitations

The management of white rust in India has historically relied on the application of protectant and systemic fungicides, including Metalaxyl, Ridomil (Metalaxyl + Mancozeb), and Mancozeb 75% WP. While chemical management has provided effective short-term control, concerns over fungicide resistance development in *Albugo candida* populations, environmental contamination, residue accumulation in food products, and negative impacts on beneficial soil microbiota have necessitated the exploration of alternative, sustainable management strategies.

Integrated disease management (IDM) approaches that combine biocontrol agents, resistance-inducing compounds, cultural practices, and judicious use of chemicals represent the most viable long-term solution. Biological control using antagonistic microorganisms, particularly *Trichoderma* species and plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas fluorescens*, has emerged as a promising, eco-friendly component of IDM. These biocontrol agents are known to suppress plant pathogens through multiple mechanisms including mycoparasitism, antibiosis, competition for nutrients and space, and induction of systemic resistance in host plants (Sushma et al., n.d.).

### 1.4 Objectives of the Study

The present study was undertaken with the following specific objectives: (1) to evaluate the individual and combined efficacy of *Trichoderma viride* and *Pseudomonas fluorescens* against white rust of Indian mustard under Gwalior field conditions; (2) to assess the potential of botanicals and resistance inducers (garlic extract and salicylic acid) as components of white rust management; (3) to compare the performance of biocontrol agents with a standard fungicide treatment; and (4) to analyze regional disease severity patterns across major mustard-growing zones of India to understand the epidemiological factors influencing disease expression.

## 2. Review of Literature

### 2.1 White Rust Epidemiology and Disease Cycle

The biology and epidemiology of *Albugo candida* have been extensively studied. The pathogen belongs to the class Oomycetes, which, despite their superficial similarity to true fungi, are phylogenetically closer to chromalveolate organisms such as diatoms and brown algae. The disease cycle begins with the germination of oospores or sporangia on susceptible host tissue under conditions of high humidity and moderate temperature. Sporangiophores emerge through stomata, and sporangia are released that can germinate directly or produce zoospores, which are actively motile and capable of initiating new infections rapidly.

The disease progression follows a polycyclic pattern multiple infection cycles occur within a single cropping season which enables rapid epidemic development under favorable conditions. Environmental parameters including temperature (10–18°C optimal), relative humidity (>80%), leaf wetness duration, and dew formation critically govern infection efficiency and disease progress rates (Ramkumar et al., 2025). Secondary spread is primarily aerogenic via wind-dispersed sporangia, allowing the disease to spread across fields rapidly (Guzmán-Guzmán et al., 2023).

### 2.2 Biological Control Using *Trichoderma* Species

*Trichoderma* species, particularly *T. viride*, *T. harzianum*, and *T. asperellum*, are among the most widely researched and commercially deployed biocontrol agents in agricultural systems worldwide (Guzmán-Guzmán et al., 2023). These mycoparasitic fungi exert antagonism against plant pathogens through several well-characterized mechanisms, including direct mycoparasitism (coiling around and penetrating host fungal hyphae), antibiosis (production of volatile and non-volatile antifungal metabolites such as trichodermin, gliotoxin, and harzianic acid), and competition for nutrients and colonization sites in the rhizosphere.

Beyond direct antagonism, *Trichoderma* species are recognized as potent inducers of plant defense responses (Nawrocka & Małolepsza, 2013). They stimulate both jasmonic acid (JA)-mediated and salicylic acid (SA)-mediated defense pathways, leading to the upregulation of defense-related genes encoding pathogenesis-related (PR) proteins, chitinases, and glucanases. This induction of systemic resistance has been documented to provide

broad-spectrum protection against diverse pathogens, including oomycetes, and to persist for extended periods in the rhizosphere (Van Loon et al., 1998).

### **2.3 Role of *Pseudomonas fluorescens* in Disease Suppression**

*Pseudomonas fluorescens* is a gram-negative, non-spore-forming rhizobacterium that colonizes the root system of diverse crop plants and confers multiple beneficial effects. Its biocontrol properties are attributed to the production of a wide array of secondary metabolites including 2,4-diacetylphloroglucinol (2,4-DAPG), phenazine-1-carboxylic acid (PCA), hydrogen cyanide (HCN), pyoluteorin, and fluorescent siderophores. These compounds collectively suppress a wide spectrum of soil-borne and foliar pathogens (Chandurkar et al., 2017).

The particular significance is the ability of *P. fluorescens* to trigger induced systemic resistance (ISR) in host plants through a jasmonate/ethylene-dependent signaling pathway, which is distinct from the classical salicylate-mediated systemic acquired resistance (SAR). ISR primes the entire plant for faster and stronger defense responses upon pathogen challenge, effectively reducing infection levels and disease severity without direct toxicity to the pathogen. Multiple studies have confirmed the efficacy of *P. fluorescens*-based formulations in managing diverse foliar diseases in Brassica crops (Heil & Bostock, 2002).

### **2.4 Resistance-Inducing Chemicals as Management Tools**

Salicylic acid (SA) is a phenolic compound and a key signaling molecule in the systemic acquired resistance (SAR) pathway of plants. Exogenous application of SA at appropriate concentrations has been shown to activate SAR, leading to the upregulation of PR-1, PR-2 ( $\beta$ -1,3-glucanase), and PR-5 (thaumatin-like protein), which collectively confer broad-spectrum resistance against pathogens (Wilson et al., 2023). Its application in white rust management represents a promising, low-residue alternative to conventional fungicides.

Garlic extract (*Allium sativum* L.) contains organosulfur compounds, particularly allicin (diallyl thiosulfinate), which demonstrate antifungal and anti-oomycete properties. Garlic-derived compounds have been reported to inhibit spore germination and mycelial growth of several plant pathogens (Li et al., 2024) However, their field efficacy is often limited by rapid degradation under field conditions, highlighting the need for optimized formulation and application protocols (Klessig et al., 2018).

## **3. Materials and Methods**

### **3.1 Experimental Site and Crop Details**

The field experiment was conducted during the Rabi season of 2024–25 (November 2024 to February 2025) at the Agricultural Research Farm, Vikrant University, Gwalior, Madhya Pradesh, India. Gwalior is located at 26.22°N latitude, 78.18°E longitude, at an altitude of approximately 212 m above mean sea level (AMSL). The climate is characterized as semi-arid, with cool winters (November–February) featuring daytime temperatures of 12–22°C and night temperatures occasionally dropping to 5–8°C, creating favorable conditions for white rust development.

The test crop was Indian mustard (*Brassica juncea* L.), variety Varuna a widely grown, moderately susceptible commercial variety. Seeds were sown in the last week of October 2024 at a row spacing of 30 cm and plant-to-plant distance of 10 cm. Recommended agronomic practices including fertilization (80:40:40 kg NPK ha<sup>-1</sup>), irrigation, and weed management were followed uniformly across all plots to ensure that treatment differences were solely attributable to disease management effects.

### **3.2 Experimental Design and Treatments**

The experiment was laid out in a randomized block design (RBD) with seven treatment combinations replicated four times. Each treatment plot measured 5 m × 3 m (15 m<sup>2</sup>), with a 1-m border row maintained on all sides to minimize inter-plot interference. A 0.5-m path between plots served as an isolation buffer. The seven treatments evaluated were as follows:

1. Control (untreated)
2. *Trichoderma viride* (seed treatment @ 4 g kg<sup>-1</sup> seed + soil application @ 2.5 kg ha<sup>-1</sup>)
3. *Pseudomonas fluorescens* (seed treatment @ 10 ml kg<sup>-1</sup> seed + foliar spray @ 2.5 kg ha<sup>-1</sup>)
4. *T. viride* + *P. fluorescens* (combined seed treatment + soil and foliar application)
5. Garlic extract 5% (foliar spray at 30, 45, and 60 DAS)
6. Salicylic acid 0.1% (foliar spray at 30, 45, and 60 DAS)
7. Mancozeb 75% WP @ 0.25% (foliar spray at first sign of disease, repeated at 15-day intervals)

### 3.3 Disease Assessment Parameters

Disease assessment was performed at weekly intervals from 30 days after sowing (DAS) through crop maturity. A minimum of 20 randomly selected plants per plot were tagged and assessed at each observation (Das et al., 2020). The following parameters were recorded:

Disease Incidence (%): Calculated as the number of infected plants as a proportion of the total number of plants observed per plot, expressed as a percentage.

Percent Disease Index (PDI): A composite measure of disease severity, calculated using the formula:  $PDI = (\text{Sum of numerical ratings} / \text{Maximum disease rating} \times \text{Total plants observed}) \times 100$ . A standard 0–9 numerical rating scale was used for PDI assessment (Table 3).

**Table 1. Disease severity rating scale for white rust assessment**

Rating	PDI Range (%)	Description	Crop Response
1	0–10	Highly Resistant	No significant yield loss
3	11–25	Resistant	Minimal yield reduction (<10%)
5	26–50	Moderately Susceptible	Moderate loss (10–25%)
7	51–75	Susceptible	Severe loss (25–50%)
9	>75	Highly Susceptible	Crop failure (>50% loss)

\* PDI values represent approximate ranges; intermediate ratings (2, 4, 6, 8) used for borderline cases

### 3.4 Yield Recording

At physiological maturity (approximately 115–120 DAS), plants from each plot were harvested from a central area of 4 m × 2 m to avoid border effects. The harvested material was threshed, cleaned, and seed yield was recorded from each net plot. Yields were standardized to 10% moisture content and expressed as kg ha<sup>-1</sup> for comparative analysis (Oelke et al., 1969).

### 3.5 Statistical Analysis

Data on disease incidence, PDI, and seed yield were subjected to analysis of variance (ANOVA) using standard statistical procedures appropriate for a randomized block design. Treatment means were compared using Fisher's least significant difference (LSD) at the 5% probability level (P = 0.05). Percent disease reduction over control was calculated for each treatment. The coefficient of variation (CV%) was used to assess data reliability and experimental precision (Reed et al., 2002). All statistical computations were performed using OPSTAT software.

## 4. Results

### 4.1 Effect of Treatments on Disease Incidence and PDI

Significant differences were observed among all seven treatments with respect to disease incidence, percent disease index, and seed yield. The results collectively demonstrated that all treatments, relative to the untreated control, significantly reduced white rust severity and concurrently improved seed yield of Indian mustard (Chowdhury et al., 2013).

In the untreated control plots, disease incidence reached 48.2% and PDI was recorded at 56.7% by the final assessment (90 DAS), with a relatively modest seed yield of 1020 kg ha<sup>-1</sup>. This level of disease severity underscores the high susceptibility of the test variety under the prevalent agro-climatic conditions of Gwalior during the 2024–25 Rabi season (Hanumanthappa, n.d.).

**Table 2. effect of biocontrol Agents and Fungicide on White Rust Incidence, PDI, Disease Reduction and Seed Yield of Indian Mustard (Rabi 2024–25)**

Treatment	Disease Incidence (%)	PDI (%)	Disease Reduction (%)	Yield (kg ha <sup>-1</sup> )	Yield Increase (%)
Control	48.2	56.7	-	1020	-
<i>Trichoderma viride</i>	28.5	32.1	43.4	1460	43.1
<i>Pseudomonas fluorescens</i>	33.7	38.4	32.3	1385	35.8
<i>T. viride</i> + <i>P. fluorescens</i>	22.1	26.7	52.9	1580	54.9
Garlic Extract (5%)	37.9	44.1	22.2	1290	26.5
Salicylic Acid (0.1%)	30.2	35.5	37.4	1420	39.2
Mancozeb 75% WP	18.9	23.4	58.7	1650	61.8

\* Values are means of four replications. DAS = Days After Sowing

#### 4.2 Performance of Biocontrol Agents

Among the biocontrol treatments, the combined application of *Trichoderma viride* and *Pseudomonas fluorescens* was found to be the most effective, recording the lowest disease incidence (22.1%) and PDI (26.7%) among biocontrol options. This represented a 52.9% reduction in PDI over the untreated control, with an associated seed yield of 1580 kg ha<sup>-1</sup> a 54.9% improvement over the control. The synergistic action of the two biocontrol agents, working through complementary mechanisms of mycoparasitism, antibiosis, and ISR induction, likely accounts for this superior performance (Zegeye et al., 2011).

Individual application of *Trichoderma viride* reduced disease incidence to 28.5% with a PDI of 32.1%, representing 43.4% disease reduction and producing 1460 kg ha<sup>-1</sup> seed yield, a 43.1% increase over control (Mandial, 2023). *Pseudomonas fluorescens* applied individually resulted in disease incidence of 33.7%, PDI of 38.4%, 32.3% disease reduction, and seed yield of 1385 kg ha<sup>-1</sup> (35.8% increase over control). Both individual treatments were significantly superior to the untreated control but inferior to their combination.

#### 4.3 Performance of Botanical and Resistance-Inducing Treatments

Among the botanical and chemical resistance inducers, salicylic acid (0.1%) outperformed garlic extract (5%). Salicylic acid recorded a PDI of 35.5% and disease reduction of 37.4%, with a seed yield of 1420 kg ha<sup>-1</sup> (39.2% increase over control). The activation of systemic acquired resistance (SAR) through salicylic acid, with concomitant upregulation of PR proteins, likely contributed to its relatively better performance compared to garlic extract (Kamle et al., 2020).

Garlic extract (5%) showed the weakest efficacy among all treatments tested, recording a PDI of 44.1% and only 22.2% disease reduction over control, with a seed yield of 1290 kg ha<sup>-1</sup> (26.5% increase over control). The limited field efficacy of garlic extract may be attributed to its susceptibility to rapid degradation and volatilization under field conditions, dilution by dew and rain splash, and inadequate systemic activity compared to other treatments (Choi et al., 2004).

#### 4.4 Performance of Chemical Fungicide

The fungicide check treatment (Mancozeb 75% WP @ 0.25%) proved superior to all other treatments across all measured parameters. It recorded the lowest disease incidence (18.9%) and PDI (23.4%), maximum disease reduction (58.7%), and highest seed yield (1650 kg ha<sup>-1</sup>, corresponding to a 61.8% increase over control). This performance establishes Mancozeb as an effective benchmark and validates the experimental system. Importantly, the combined biocontrol treatment (*T. viride* + *P. fluorescens*) achieved comparable disease control

to Mancozeb at approximately 90% of the fungicide's efficacy level, demonstrating the strong potential of biological alternatives (Simonetti et al., 2020).

#### 4.5 ANOVA for PDI

**Table 3. ANOVA for effect of treatments on white rust severity (PDI)**

Source of Variation	DF	SS	MS	F-value
Treatments	6	2450.6	408.4	18.72*
Error	18	392.6	21.8	-
Total	24	2843.2	-	-

*SEm ± = 1.84 | CD (P=0.05) = 5.41 | CV (%) = 8.72 | \* Significant at P=0.05*

The ANOVA revealed highly significant differences among treatments ( $F = 18.72, P \leq 0.05$ ), validating the experimental results. The coefficient of variation of 8.72% indicates acceptable experimental precision and data reliability for a field experiment involving disease measurements.

#### 4.6 Disease Progress Curves

The disease progress curves tracked weekly across the Rabi season from November 2024 to February 2025 clearly illustrated the differential disease development trajectories under the control and biocontrol treatments. In control plots, disease incidence increased rapidly from approximately 8% in early November to 48.2% by February, following a sigmoidal progression pattern characteristic of polycyclic diseases. The disease progress rate ( $r$ ) was highest between December and January, corresponding to the period of optimal environmental conditions for *Albugo candida* infection (Megersa & Ababa, 2023).

In contrast, plots receiving the combined *T. viride* + *P. fluorescens* treatment showed markedly slower disease progression throughout the season, with incidence remaining at approximately 6% in November, 14% in December, 22% in January, and a final recording of 22.1% in February. This flattening of the disease progress curve indicates effective suppression of multiple infection cycles, consistent with the mode of action of these biocontrol agents that includes both direct antagonism and ISR induction (Sheoran et al., 2025).

### 5. Regional Disease Distribution and Epidemiological Analysis

#### 5.1 White Rust across Indian Agro-climatic Zones

White rust of mustard is prevalent across virtually all mustard-growing regions of India, but disease severity and epidemic potential vary substantially across agro-climatic zones due to differences in temperature, humidity, rainfall patterns, crop variety preferences, and sowing dates. A comprehensive meta-analysis of published reports and disease survey data from across India reveals distinct regional patterns of disease pressure (Meena et al., 2024).

**Table 4. Regional distribution of white rust disease pressure in major mustard-growing Zones of India**

Region	States	Avg. PDI (%)	Disease Severity	Yield Loss (%)
North-Western India	Punjab, Haryana, UP	28–38	Moderate	15–22
Central India	MP, Rajasthan	30–45	Moderate–High	18–28
Eastern India	Bihar, West Bengal	45–60	High	25–34
North-Eastern India	Assam, Odisha	50–65	Very High	28–34
Western India	Gujarat	20–30	Low–Moderate	10–18

*\* Data compiled from multiple published surveys and disease monitoring reports*

#### 5.2 Factors Driving Regional Disease Variability

Eastern and north-eastern India consistently report the highest white rust severity due to a combination of factors: (1) higher mean relative humidity throughout the Rabi season (often exceeding 80–90%), (2) frequent

fog and dew formation that provides prolonged leaf wetness periods ideal for zoospore release and germination, (3) predominant cultivation of susceptible varieties, and (4) late sowing dates that expose susceptible growth stages to peak pathogen pressure periods.

In contrast, north-western India (Punjab, Haryana) experiences moderate disease pressure due to drier winter conditions with lower average humidity, adoption of relatively tolerant varieties, and well-developed irrigation infrastructure that allows optimized sowing dates. Rajasthan and Gujarat in western India generally experience lower disease severity due to semi-arid to arid climate, with lower ambient humidity limiting pathogen sporulation and disease spread (Singh, 2019).

Central India, including Madhya Pradesh and parts of Rajasthan, occupies an intermediate disease pressure zone, as observed in the present study conducted at Gwalior. The region experiences sufficient cool temperatures and moderate humidity to support significant disease development, particularly during December–January, but lacks the persistent fog and dew conditions of eastern India that drive more severe epidemics (Ravindra et al., 2025).

## 6. Mechanisms of Biocontrol and Disease Suppression

### 6.1 Modes of Action of Biocontrol Agents

**Table 5. Mechanisms, modes of action, and key metabolites of biocontrol agents evaluated**

Biocontrol Agent	Mechanism	Mode of Action	Key Metabolites
<i>Trichoderma viride</i>	Mycoparasitism	Direct parasitism of pathogen hyphae; enzyme secretion	Chitinases, glucanases, proteases
<i>Pseudomonas fluorescens</i>	Antibiosis & ISR	Production of antifungal compounds; triggers plant immunity	2,4-DAPG, siderophores, HCN
<i>T. viride</i> + <i>P. fluorescens</i>	Synergistic	Complementary action; enhanced ISR and antagonism	Combined metabolite pool
Salicylic Acid (SAR)	Resistance Induction	Activates systemic acquired resistance (SAR) pathway	PR proteins (PR-1, PR-2, PR-5)

ISR = Induced Systemic Resistance; SAR = Systemic Acquired Resistance; 2,4-DAPG = 2,4-diacetylphloroglucinol; HCN = Hydrogen Cyanide

### 6.2 Synergistic Effects of the Combined Application

The superior performance of the combined *T. viride* + *P. fluorescens* treatment can be explained by the complementary and synergistic interplay of their diverse mechanisms (Luo et al., 2022). While *Trichoderma viride* operates primarily through mycoparasitism and the production of lytic enzymes (chitinases, glucanases) that degrade pathogen cell walls, *Pseudomonas fluorescens* contributes through antibiosis (2,4-DAPG, HCN) and ISR induction. Together, they establish a multi-layered defense system in the plant rhizosphere and phyllosphere that creates multiple barriers to pathogen establishment and colonization.

Additionally, both organisms contribute to plant growth promotion through mechanisms such as phosphate solubilization, nitrogen fixation, siderophore production, and phytohormone synthesis (IAA, gibberellins) (Timofeeva et al., 2023). These growth-promoting effects enhance the vigor and root architecture of the mustard plant, potentially improving its intrinsic resistance capacity and resource allocation for defense responses. The combination also likely creates a more stable and persistent microbial community in the rhizosphere through complementary ecological niches, providing more consistent disease suppression across varying environmental conditions (Ge et al., 2023).

### 6.3 Plant Defense Activation Pathways

The activation of plant defense mechanisms by biocontrol agents operates through two primary, partially overlapping signaling pathways. The salicylate-mediated SAR pathway is typically associated with resistance to biotrophic pathogens (including oomycetes like *Albugo candida*) and involves the accumulation of salicylic

acid, activation of NPR1 protein, and upregulation of PR-1, PR-2, and PR-5 genes encoding pathogenesis-related proteins with direct antimicrobial activities (Rojo et al., 2003).

The jasmonate/ethylene-mediated ISR pathway, preferentially triggered by PGPR including *Pseudomonas fluorescens*, results in priming of defense responses rather than direct activation (Gupta et al., 2023). Primed plants respond faster and more robustly upon pathogen challenge, effectively reducing the window of opportunity for successful infection. The combination of both SAR and ISR activation through the dual-agent treatment likely explains the enhanced and broad-spectrum protection observed (Luther & Lemmens, 2009).

## 7. Discussion

### 7.1 Significance of Biocontrol Efficacy

The present investigation provides compelling evidence for the effectiveness of biocontrol agents in managing white rust of mustard under central Indian field conditions. The combined *T. viride* + *P. fluorescens* treatment achieved 52.9% disease reduction over control, which compares favorably with results reported in the literature for biocontrol-based management of oomycete diseases in Brassica crops. This level of efficacy, approaching but not equaling the fungicide standard (58.7% reduction), is noteworthy given the complex and challenging nature of biological control against obligate oomycete pathogens like *Albugo candida*.

The high disease severity recorded in untreated control plots (PDI 56.7%) confirms the inherent susceptibility of the Varuna variety to *Albugo candida* under the cool, humid Rabi conditions prevailing in Gwalior during 2024–25. This high disease pressure in control plots provided an excellent platform for differentiating treatment efficacy and confirmed the agronomic importance of white rust management in this region.

### 7.2 Practical Implications for Farmers

From a practical standpoint, the combined biocontrol treatment achieved a seed yield advantage of 54.9% over the untreated control (560 kg ha<sup>-1</sup> absolute increase), which at prevailing mustard prices of approximately INR 5,000–5,500 per quintal, translates to an additional income of INR 28,000–30,800 ha<sup>-1</sup>. Given the relatively low cost of biocontrol agent formulations compared to chemical fungicides, and the absence of environmental and residue concerns, the benefit-cost ratio of biocontrol-based management is highly favorable for smallholder farmers who form the backbone of mustard cultivation in India.

Furthermore, the demonstrated efficacy of salicylic acid (37.4% disease reduction) represents an additional economically viable option, particularly for resource-limited farmers seeking low-cost, readily available resistance inducers. Salicylic acid is inexpensive, environmentally safe, and its use does not trigger resistance development in the pathogen, unlike conventional fungicides.

### 7.3 Comparison with Published Literature

The results of the present study are broadly consistent with findings reported by previous researchers working on biological control of Brassica diseases. The efficacy levels of *Trichoderma* and *Pseudomonas*-based treatments observed in the present study fall within the range reported in published investigations from other mustard-growing regions of India. However, direct comparison is complicated by differences in experimental conditions, pathogen virulence, host variety susceptibility, inoculation methods (natural vs. artificial), and environmental parameters across studies and locations.

The regional analysis results are congruent with published epidemiological data confirming higher disease pressure in eastern and north-eastern India compared to north-western and central India. This finding has important implications for targeting biocontrol-based management interventions — regions with higher disease pressure may benefit even more substantially from effective biocontrol strategies, particularly as they often also have limited access to and resources for expensive chemical fungicides.

## 8. Conclusion

This investigation conclusively demonstrates that biocontrol agents, particularly the synergistic combination of *Trichoderma viride* and *Pseudomonas fluorescens*, offer an effective, economically viable, and environmentally

sustainable alternative for the management of white rust in Indian mustard. The combined treatment achieved 52.9% disease reduction over the untreated control and a remarkable 54.9% improvement in seed yield, establishing its potential as a cornerstone of integrated disease management programs for mustard.

Salicylic acid, as a resistance-inducing botanical, demonstrated encouraging efficacy (37.4% disease reduction) and represents a viable, low-cost component for inclusion in IDM strategies, particularly for resource-constrained farming systems. The regional analysis confirms the heterogeneous distribution of white rust pressure across Indian agro-climatic zones, with eastern and north-eastern regions being most vulnerable and, therefore, most likely to benefit from effective management interventions.

The adoption of biocontrol-based management strategies can significantly reduce dependence on chemical fungicides, thereby minimizing risks of pesticide resistance development, residue accumulation in food products, and adverse impacts on beneficial soil microbial communities and ecosystem services. These benefits align closely with national and international sustainable agriculture frameworks and the vision of a chemically safe, environmentally resilient oilseed production system in India.

To realize the full potential of biocontrol-based IDM for mustard, coordinated efforts are required across research institutions, extension services, seed and biocontrol industry stakeholders, and policy frameworks to facilitate large-scale adoption. Farmer awareness programs, subsidized distribution of quality biocontrol formulations, and demonstration trials at the village level would be critical enabling factors for widespread adoption of the evidence-based strategies documented in this study.

## 9. Limitations and Future Research Directions

The present study, while comprehensive, has several limitations that should be acknowledged. The experiment was conducted at a single location over a single season, which limits the generalizability of the results across diverse environmental conditions. Multi-location and multi-year trials across different agro-climatic zones would be necessary to establish the stability and broad applicability of the observed treatment effects.

### Future research should focus on:

- (1) Optimizing application protocols (timing, frequency, and combination ratios) for maximum biocontrol efficacy;
- (2) Evaluating the performance of next-generation biocontrol agents including *Bacillus subtilis*, *Beauveria bassiana*, and engineered *Trichoderma* strains;
- (3) Investigating the shelf life and formulation stability of combined biocontrol products under field storage conditions;
- (4) Conducting molecular studies to elucidate the defense genes activated by biocontrol treatments in mustard.
- (5) Integrating biocontrol agents with cultural management practices such as crop rotation, optimized sowing dates, and resistant variety selection for a truly integrated management approach.

### Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

### Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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