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Research Article

Impact of Foliar Application of Systemic Fungicides on Yield Attributes and ACI Against Stripe Rust of Wheat

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Abstract

Wheat is a vital global cereal crop, but its productivity and nutritional quality are severely threatened by stripe rust, a devastating fungal disease caused by *Puccinia striiformis f. sp. tritici*. When genetic resistance in cultivars fails, fungicide application is critical for protecting yields. A study conducted at Khalsa College Amritsar (2022-24) evaluated the efficacy of various fungicides against stripe rust using a Randomized Block Design. Treatments were compared to a control (cultivar PBW-343) based on disease severity, the Average Coefficient of Infection (ACI), and yield parameters including grain yield and 1000-grain weight. Under artificial inoculation, Nativo 75 WG (trifloxystrobin + tebuconazole) applied twice at 15-day intervals recorded the highest grain yield (49.41 q/ha) and 1000-grain weight (43.79 g), increasing yield by 60.95% over control. Custodia, Fusion Top, and Tilt followed, while Opera (pyraclostrobin + epoxiconazole) showed the lowest efficacy (45.68 q/ha, 41.17 g). Nativo also had the lowest ACI (1.6%), compared to the control (54.34%). Other treatments-Custodia (2.4%), Fusion Top (4.65%), Tilt (8.4%), and Opera (11.6%) were statistically superior to control. Systemic fungicides like Custodia, Nativo, Tilt, Opera, and Fusion Top, with diverse modes of action (e.g., DMIs, QoIs), effectively suppressed the disease. These findings highlight their role in managing stripe rust and safeguarding wheat productivity.

Keywords

ACI, Stripe Rust, Systemic Fungicides, Wheat, Yield Attributes

1. Introduction

Wheat (*Triticum aestivum L.*) is a foundational cereal crop, measured by its vast cultivation area, high production volume, and the number of farmers it employs [1] This domesticated grass of the Poaceae family is a hexaploid species (2n=42) [2] that offers a balanced diet rich in protein, vitamins, and carbohydrates. Its economic importance in India is immense, serving as a primary nutritional source in the north and north-west [3]. Meeting global demand requires increased production; currently, wheat is planted on one-sixth of the world's arable land [4]. In India, the 2023-24 harvest spanned 341.57 lakh hectares, producing 110.554 million tons. Punjab state alone accounted for 35.26 lakh hectares and an output of 17.78 million tons [5]. A range of biotic and abiotic factors threaten wheat yields, with diseases caused by pathogens like fungi, bacteria, and viruses posing a major threat [6]. The most economically destructive diseases are the rusts (leaf, stripe, and stem rust), which are considered serious global threats to production [7]. These pathogens cause significant losses by developing pustules on leaves, which reduce the green leaf area available for photosynthesis. This damage

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directly leads to a decline in both grain yield and quality, often resulting in severe production losses [8]. The economic impact of wheat rust is severe, with stem rust capable of causing total (100%) crop loss and stripe rust losses of up to 60% [9]. These diseases are identified by the appearance of yellow, powdery spore masses (pustules) that form stripes on leaves and other aerial plant structures. The development of stripe rust epidemics is highly dependent on weather; the pathogen proliferates rapidly under conditions of low temperature, high relative humidity, and rainfall, while its development is suppressed by high maximum temperatures [10]. A key feature of its biology is its ability to be transported aerially over long distances, which facilitates sudden and widespread outbreaks in conducive environments [11]. The primary damage mechanism is the disruption of the plant's photosynthetic system by the pustules, leading to significant reductions in grain weight and overall quality [12, 13]. When host plant resistance is unavailable or has been overcome by evolving pathogen populations, fungicide application becomes a critical strategy for managing stripe rust. Although the use of susceptible cultivars often necessitates fungicide intervention to prevent epidemics, their effectiveness is highly dependent on the applied concentration [14, 15]. The efficacy of specific active ingredients-including epoxiconazole, pyraclostrobin, and propiconazole-has been demonstrated to be directly correlated with their dosage rates [15]. Despite concerns about cost and environmental impact, fungicides remain an efficient and routine practice in many countries for preventing epidemics and preserving both yield and grain quality, especially in high-value wheat crops [16]. This is particularly mandatory under high inoculum pressure to achieve optimum yield [17]. The emergence of novel, virulent pathotypes that break down resistance in commercial cultivars further underscores the necessity of fungicide intervention as a key component of integrated disease management [18].

The primary fungicides deployed against wheat stripe rust belong to the triazole (DMI, Group 3) and strobilurin (QoI, Group 11) chemical classes [19]. Triazoles, known for their systemic activity as they are absorbed and translocated within the plant, are commonly used. However, combination products integrating a triazole with a strobilurin have become increasingly prevalent and are widely approved for control [20]. Strobilurins themselves are highly effective and represent some of the most extensively used agricultural fungicides globally. The success of any fungicide application is critically dependent on correct timing, aligning with both the susceptible stage of host development and the initial establishment of the pathogen [21]. Experimental evidence consistently demonstrates that sprayed plots yield significantly more than unsprayed controls, with the application interval being a key factor in reducing disease severity, as measured by metrics like the Average Coefficient of Infection (ACI) [22, 23]. Thus, the timely and precise use of these fungicides is a vital strategy for safeguarding crop productivity.

In Punjab state of India, yield losses up to 68.3 per cent have been reported [24]. In addition to the deliberate attempts to generate more reliable resistant cultivars, disease management through fungicide administration is conducted, particularly where non-recommended susceptible cultivars are cultivated [25]. The literature clearly shows that previously resistant varieties became susceptible over time. As a result, researchers must get a better understanding of the chemicals that are effective against the stripe rust pathogen to recommend appropriate and effective toxicants when chemical fungicide management is urgently required. Given all of this, it is worthwhile to assess the comparative efficacy of newer available fungicides to determine the optimal fungicide. Present investigations were undertaken to study the efficacy of new molecules fungicides against stripe rust of wheat in relation with yield attributes *viz.*, Grain yield and thousand grain weight. In the Punjab state of India, stripe rust has been responsible for substantial yield losses, documented to reach up to 68.3% [24]. The literature clearly shows that previously resistant varieties became susceptible over time. As a result, researchers must get a better understanding of the chemicals that are effective against the stripe rust pathogen to recommend appropriate and effective toxicants when chemical fungicide management is urgently required. Given all of this, it is worthwhile to assess the comparative efficacy of newer available fungicides to determine the optimal fungicide. Present investigations were undertaken to study the efficacy of new molecules fungicides against stripe rust of wheat in relation with yield attributes *viz.*, Grain yield and thousand grain weight.

2. Material and Methods

2.1. Study Area

This research was carried out in Chheharta, Amritsar, an area representative of the major wheat-producing zones in the Majha region of Punjab, India, and a known hot spot for stripe rust epidemics. The experimental site, situated at 31.6335 °N latitude and 74.8358 ° E longitude (218 meters above sea level), features a hot semi-arid to subtropical humid climate characterized by a wide temperature range, from 1-2 ℃ in winter to 40-44 ℃ in summer.

2.2. Description of Experimental Setup and Design

The experiment was arranged in a Randomized Block Design (RBD) with three replications to evaluate fungicide efficacy. A highly susceptible wheat cultivar, PBW 343, was uniformly sown across 18 plots, each measuring 3×2 m^2. This variety, known for its consistent susceptibility to stripe rust in previous screenings, was also planted as a susceptible check surrounding the experimental plots to promote uniform inoculum spread. All agronomic management practices, including field preparation, fertilizer application, weed control, and irrigation-were carried out in accordance with the recommended package of practices provided by Punjab Agricultural University, Ludhiana [52]. The experimental field featured clay-loam soil, which was prepared to a fine tilth through primary and secondary tillage operations. To promote optimal germination, light irrigation was applied immediately after sowing. Standard agronomic practices, including weeding, hoeing, and plant protection measures, were implemented once a uniform crop stand was established. Five fungicidal treatments were evaluated alongside an untreated control (tap water spray): azoxystrobin + tebuconazole (Custodia 320 SC), trifloxystrobin + tebuconazole (Nativo 75 WG), pyraclostrobin + metconazole (Opera 18.3 SE), propiconazole (Tilt 25 EC), and a premix of azoxystrobin (18.20%) + difenoconazole (11.40%) (Fusion Top). Two foliar sprays were applied for each treatment using a hand-operated sprayer. The first application was timed at the onset of disease, when approximately 5% of leaves exhibited initial symptoms, followed by a second spray 15 days later. Disease severity was assessed on randomly selected plants using the modified Cobb's scale [26, 27], and the resulting data were used for subsequent analysis.

Table 1. *Description of systemic fungicides tested against Stripe rust of Wheat.*

Treatment	Active ingredient	Trade name	Brand name	Formulations	Dose (in %)
T1	azoxystrobin+ tebuconazole	Custodia 320 SC	Adama	SC	0.1%
T2	trifloxystrobin (50%)+ tebuconazole (25%)	Nativo 75 WG	Bayer	WG	0.1%
T3	propiconazole 25 EC	Tilt 25 EC	Crystal	EC	0.1%
T4	pyraclostrobin (13.5%) + epoxiconazole (5%)	Opera 18.3 SE	AgCelence	SE	0.1%
T5	azoxystrobin (18.20%) + difenoconazole (11.40%)	Fusion Top	Indian Agrochemicals	SC	0.1%

SC-Aqueous suspension concentrates; WG-Wettable granules; SE-Aqueous suspension; EC-Emulsifiable concentrate

2.3. Induction of Disease Epiphytotic

Inoculation was conducted when the crop was 40 days old. The inoculum consisted of a mixture of *Puccinia striiformis* f. sp. *tritici* pathotypes (46S119, 110S199, 238S119, 110S84, and 47S103), which was procured from the Regional Station of the Indian Institute of Wheat and Barley Research (IIWBR), Flowerdale, Shimla, Himachal Pradesh. This pathogen mixture was used for screening wheat cultivars and evaluating fungicide efficacy against stripe rust**.** The pathotypes selected for the study were several, diversified, and highly virulent. These pathotypes are diverse and considerably different in terms of their capacity to survive major resistance genes. *P. striiformis* pathotypes were inoculated into wheat plants at the three to four leaf stage. Artificial inoculation of pathotype inoculum can be done by preparing a spore suspension i.e., mixing spore dust of pathotypes mixture in double distilled water and three to four fine mist sprays of water consisting of uredospores 1 g/litre with an approximate concentration of 10,000 spores/ml in the suspension were carried out in evening between 45-55 days after sowing. The spore suspension was sprayed with the help of small hand sprayer (sterilized with ethanol) evenly on the wheat germplasm at border lines to create natural epiphytotic conditions. The spore suspension was allowed to be applied repeatedly and if possible, immediately after a rainfall incidence to increase chances of disease establishment at the field.

Table 2. *Host response of Stripe rust on basis of rust severity.*

HOST RESPONSE	SEVERITY RANGE	RESPONSE VALUE
No Disease (O)	0	0.0
Resistant (R)	1-10	0.2
Resistant to Moderately Resistant (RMR)	10.1-20	0.3
Moderately Resistant (MR)	20.1-30	0.4
Moderately Resistant to Moderately Susceptible (MRMS)	30.1-40	0.6

HOST RESPONSE	SEVERITY RANGE	RESPONSE VALUE
Moderately Susceptible (MS)	40.1-50	0.8
Moderately Susceptible to Susceptible (MSS)	50.1-60	0.9
Susceptible (S)	More than 60	1.0

2.4. Disease Assessment, Grain Yield and Thousand Grain Weight

Upon the appearance of stripe rust symptoms, fungicide treatments were administered. Disease assessments were conducted at at 0th day, 7th day and 14th day of spraying. The Average Coefficient of Infection (ACI) was calculated by multiplying the percentage disease severity by a constant value corresponding to the host response type, according to established methods [28, 29], using the formula:

$$\text{ACI} = \text{Rust severity value} \times \text{Response value}$$

At maturity, each plot (3 x 2 m^2) was harvested and threshed individually to record grain yield data in kilograms, which was finally converted to hectare base and presented as quintal per hectare. The samples drawn from harvested grains were used to calculate thousand grain weight.

Percentage-based disease severity was determined by assigning estimated values from a standardized interval scale [30].

$$\text{Disease Severity (\%)} = \frac{\text{Area of plant tissue infected}}{\text{Total area of the plant part examined}} \times 100$$

Percent increases in yield by various treatments were calculated by using the formula [30], Where Y I=Percent increase in yield; Y1=Yield of treated plot; Y2= Yield of Untreated plot (control).

$$\text{Y I} = \frac{\text{Y1} - \text{Y2}}{\text{Y2}} \times 100$$

1000-Grain weight: Thousand fully developed grains from the samples drawn by harvested grains were used to calculate Average (g) thousand grain weight [15] (Sharma *et al* 2016).

2.5. Statistical Analysis

The data obtained from the field experiment, arranged in a Randomized Block Design (RBD), were analyzed using appropriate statistical methods as outlined by [31]. Critical Difference (CD) and Coefficient of Variation (CV) were calculated at a 5% significance level to evaluate differences among treatments. Treatment means were compared using Duncan's Multiple Range Test (DMRT) executed in IBM SPSS Statistics version 22 [32].

3. Results and Discussion

3.1. Effect on Average Coefficient of Infection

The Average Coefficient of Infection (ACI) served as a key parameter for evaluating the comparative bio-efficacy of foliar-applied fungicides Chemical management against stripe rust were conducted under artificial inoculation condition, showed that all the treatments were significantly effective and attained lower ACI value compared with control (54.34%) during the 2023-24 cropping season. Each fungicide was sprayed twice at 15 days intervals. Among the five fungicides tested, Nativo 75 WG (tebuconazole 50% + trifloxystrobin 25% WG) applied at 0.1% concentration demonstrated the highest efficacy, achieving the lowest ACI (1.6%). It was followed by Custodia 320 SC (ACI = 2.4), Fusion Top (ACI = 4.65), and Tilt 25 EC (ACI = 8.4). Opera 18.8 SE showed the highest ACI value (11.6) among the treatments, though it remained statistically superior to the control. All fungicide treatments were significantly more effective than the untreated check based on statistical analysis.

3.2. Effect on Grain Yield and Thousand Grain Weight

The various fungicide treatments significantly influenced yield attributes, specifically grain yield and thousand-grain weight. As detailed in Table 3 and Figure 1, all treatments produced significantly higher grain yields compared to the untreated control (29.43 q/ha). The highest grain yield (49.41 q/ha) was obtained from plots treated with Nativo 75 WG (tebuconazole 50% + trifloxystrobin 25% WG) applied at 0.1%, followed by Custodia 320 SC (47.38 q/ha), Fusion Top (46.68 q/ha), and Tilt 25 EC (46.24 q/ha). The lowest yield among treated plots was recorded with Opera 18.8 SE (45.76 q/ha). Similarly, the highest thousand-grain weight (43.79 g) was observed in the Nativo 75 WG treatment, followed by Custodia 320 SC (42.98 g), Fusion Top (42.23 g), and Tilt 25 EC (41.87 g). Opera 18.8 SE resulted in the lowest thousand-grain weight (41.17 g). The study demonstrated that combination fungicides, particularly Nativo 75 WG and Custodia 320 SC, were most effective, increasing yield by 67.88% and 60.95%, respectively. Fusion Top, Tilt 25 EC, and Opera 18.8 SE also substantially improved yields, with increases of 58.61%, 57.11%, and 55.48%, respectively.

Table 3. Impact of fungicides on Average coefficient of infection (Disease severity) and yield parameters against Stripe rust of Wheat under artificial inoculation during Rabi 2023-24.

Treatments	No. of sprays	ACI value*	Grain yield (q/ha)*	1000 grain weight (gram)*	Increase in yield (%)
Custodia 320 SC (azoxystrobin+ tebuconazole)	2	2.4	47.37 b+ 0.23**	42.98ab+0.13**	60.95
Nativo 75 WG (trifloxystrobin 50%+ tebuconazole 25%)	2	1.6	49.41a + 0.32**	43.79 a +0.07**	67.88
Tilt 25 EC (propiconazole 25 EC)	2	8.4	46.24c + 0.41**	41.87 c +0.4**	57.11
Opera 18.3 SE (pyraclostrobin 13.5% + epoxiconazole 5%)	2	11.6	45.76c + 0.08**	41.17 c +0.33**	55.48
Fusion Top (azoxystrobin 18.20% + difenoconazole 11.40%)	2	4.5	46.68bc+ 0.15**	42.23bc +0.27**	58.61
Control	-	54.34	29.43	33.56	-
S.E.(m)		0.027			
C.D. (ρ<0.05)		0.090	0.946	1.055	
C.V.		0.826			

ACI value: Average coefficient of infection
*Average of three replications
**Mean + SE with superscript row wise (small alphabet) differ significantly (ρ<0.05)

4. Discussion

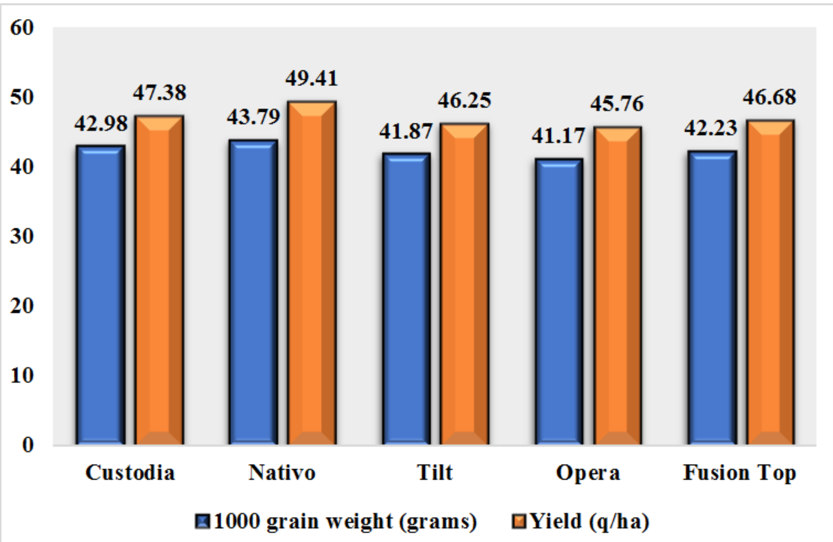

Figure 1. Impact of systemic fungicides on yield parameters against Stripe rust of Wheat under artificial inoculation during, Rabi 2023-24.

The use of resistant cultivars and chemical substances are the main methods for the management of stripe rust. Fungicide application provides an effective and practical tool for minimizing disease epidemics [33-36]. To reduce subsequent progression of the disease on the plant, fungicides should be applied at the time of disease appearance to achieve effective results [37, 38]. The effectiveness of fungicides is dependent on the growth stage of the crop and disease level at the time of application [39] (Viljanen-Rollinson *et al* 2002). The results coincide with [40] reported higher grain yield and thousand grain weight was obtained by Amistar xtra (57.03 q/ha and 45.6 g) followed by Custodia 320 SC (51.1 q/ha and 43.1%), Opera 175 SE (52.6 q/ha and 43.4%), Tilt 25 EC (55.9 q/ha and 44.5%) each @ 0.1% and Nativo 75 WG @ 0.6% (52.9 q/ha and 43.9%).

Results are in conformity with [41] recorded maximum grain yield in the plot treated with tebuconazole 50%+ trifloxystrobin 25% WG@ 0.06% over untreated control (45.22 q/ha) followed by picoxystrobin 7.05%+ propiconazole 11.7% SC@ 0.1% (44.48 q/ha), azoxystrobin 11% + Tebuconazole 18.3% w/w SC@ 0.1% (43.73 q/ha), pyroclostrobulin 113 g/l+ epoxiconazole 50 g/l SE@ 0.1% (43.00 q/ha), propiconazole 25 EC@ 0.1% (42.31 q/ha), azoxystrobin 18.2%+ cyproconazole 7.3% w/w SC@ 0.1% (41.47 q/ha), azoxystrobin 18.2%+ difenconazole 11.4% SC@ 0.1% (40.95 q/ha), tebuconazole 25 EC@ 0.1% (40.04 q/ha). Also, highest 1000 grain weight 47.43 g was again observed in tebuconazole 50%+ trifloxystrobin 25% WG@ 0.06% followed by picoxystrobin 7.05%+ propiconazole 11.7% SC@ 0.1% (46.69 g), azoxystrobin 11% + tebuconazole 18.3% w/w SC@ 0.1% (45.2 g), pyroclostrobin 113 g/l+ epoxiconazole 50 g/l SE@ 0.1% (44.42 g), propiconezole 25 EC@ 0.1% (44.20 g), azoxystrobin 18.2%+ cyproconazole 7.3% w/w SC@ 0.1% (43.34 g), azoxystrobin 18.2%+ difenconazole 11.4% SC@ 0.1% (42.95 g), tebuconazole 25 EC@ 0.1% (41.83 g) and all treatments are effective and attained lower ACI value compared with control. The least ACI (1.33%) was observed in Tebuconazole 50%+ Trifloxystrobin 25% WG@ 0.06% followed by Picoxystrobin 7.05%+ Propiconazole 11.7% SC@ 0.1%(1.67%), Azoxystrobin 11% + Tebuconazole 18.3% w/w SC@ 0.1%(2.67%), Pyroclostrobin 113 g/l+ Epoxiconazole 50 g/l SE@ 0.1% (4.67%), Azoxystrobin 18.2%+ Cyproconazole 7.3% w/w SC@ 0.1% (5.33%), Propiconezole 25 EC@ 0.1% (6.0%), Azoxystrobin 18.2%+ Difenoconazole 11.4% SC@ 0.1% (7.33%), Tebuconazole 25 EC@ 0.1%(8.33). Similarly, [22] also concluded the highest mean grain yield of 32.24 q/ha was recorded in two sprays of azoxystrobin 20%+difenconazole 12.5% SC @0.1% with mean increase in yield of 56.73% over check, followed by 32.10, 31.47, 31.18 and 29.65 q/ha yield in tebuconazole 25% EC, azoxystrobin 23.1%SC, tebuconazole 50%+ trifloxystrobin 25% w/w 75 WG and propiconazole 25%EC, with mean yield increase of 11.54, 10.91, 10.62 and 9.08 q/ha with 56.08, 53.01, 51.60 and 44.14% over the unsprayed check which also supported the present study. [42] observed that disease severity of stripe rust reached 30% in various bread wheat cultivars during the 2017-18 season and discovered that rust diseases have a substantial negative correlation with grain production. Grain production losses in susceptible cultivars could reach 100% if stripe rust infection occurs early [43]. These fungicides have different modes of action against the fungus, such as demethylation inhibitors (DMI) with triazole and imidazole chemical families and quinine outside inhibitors (Qol) with strobilurins family [44, 45]. Mixture of triazoles and strobilurins help to check both fungal growth as well as spore germination and thus prove highly effective [46]. Fungicide formulations tested in the present study are a combination of different strobilurins and triazoles, which effectively checked the fungus on wheat through mitochondrial inhibition, and sterol bio-synthesis inhibition respectively [46]. Azoxystrobin, also known as β-methoxyacrylates, is synthesised from naturally occurring strobilurins [47]. Azoxystrobin interferes with ATP synthesis by binding to the Qo site of complex III in the mitochondrion, disrupting the electron transport chain [48]. Propiconazole is derivative of triazole, a demethylated inhibitor that interferes with ergosterol biosynthesis by suppressing the oxidative removal of 14 α -methyl group from 24- methylenedihydrolanosterol in fungi [49]. Ergosterol deprivation causes plasma membrane instability, that leads to failure of fungus nutrition and eventual death [50]. Difenoconazole is the first sterol inhibitor compound, classified on basis of its mode of action. First, it inhibits sterol C-14 demethylation, which interferes with the P-450 enzyme; second, it inhibits C-14 reductase [51].

With multiple modes of action, they were able to give complete inhibition of the disease [40]. In the present study, it was found that the fungicide Tilt and fungicidal mixtures-Nativo, Custodia, Opera and Amistar top are highly effective against yellow rust in reducing the average coefficient of infection (also disease severity) and elevates the per cent grain yield and thousand grain over unsprayed plot and these fungicides may be used against this disease as a short-term alternative of resistant varieties for the multiple disease management strategies.

5. Conclusion

The current study clearly shows that in Punjab, it is difficult to grow susceptible wheat varieties without fungicide application in areas with stripe rust as a major problem. High grain yield and 1000 kernel weight recorded from plot treated with Nativo and Custodia were best comparative to other treatments. Mixture of triazoles and strobilurins help to check both fungal growth as well as spore germination and thus prove highly effective. However, further research is needed to come up with other management options to sustainable and cost effectively manage the disease under different agro-ecological regions.

Abbreviations

ACI	Average Coefficient of Infection
IIWBR	Indian Institute of Wheat and Barley Research
ICAR	Indian Council of Agricultural Research
CD	Critical Difference
CV	Coefficient of Variation
RBD	Randomized Block Design
DMRT	Duncan's Multiple Range Test
QoI	Quinone Outside Inhibitor
DMI	Demethylation Inhibitor
SC	Soluble Concentrate
EC	Emulsifiable Concentrate
SE	Aqueous Suspension
WG	Wettable Granule

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Author Contributions

Muskan Bhullar: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft

Seethiya Mahajan: Investigation, Supervision, Validation, Writing – review & editing

Data Availability Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors**.**

Conflicts of Interest

The authors declare no conflicts of interest.

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