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Research Article

Assessment of Fungicide Efficacy and Varietal Resistance to *Alternaria* Blight in Canola

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ABSTRACT

Alternaria blight, caused by *Alternaria brassicae*, is a major yield-limiting disease of canola worldwide. The identification of tolerant genotypes and effective management strategies is crucial for sustainable production. This study evaluated the response of canola genotypes to *Alternaria* blight under natural field conditions and assessed the efficacy of fungicidal treatments. Field screening revealed considerable variation in disease response among genotypes. None of the tested lines exhibited immunity or complete resistance. Four genotypes, namely Anmol Raya, Khanpur Raya, Super Raya, and AARI Canola, were categorized as moderately resistant, while the majority, including Rohi-Sarsoon, Punjab Canola, Faisal Canola, Peela Raya, and Super Canola, were highly susceptible. Disease severity ranged from 40.33% to 56.00%, with the highest in Rohi-Sarsoon (56.00%) and Punjab Canola (55.00%), and the lowest in AARI Canola (40.33%) and Super Raya (41.00%). Disease incidence followed a similar trend, ranging from 45.67% in AARI Canola to 82.33% in Faisal Canola. Fungicide trials on highly susceptible varieties (Punjab Canola and Rohi-Sarsoon) demonstrated significant reductions in disease severity. Combination treatments proved most effective, particularly Mancozeb + Metalaxyl, which reduced severity to 22% and 20% in Punjab Canola and Rohi-Sarsoon, respectively, after 21 days. Metalaxyl alone and the three-way combination of Mancozeb + Carbendazim + Metalaxyl also showed strong efficacy, whereas single fungicides, especially carbendazim, were less effective. Overall, the study highlights the absence of immune genotypes in the tested panel but identifies moderately resistant lines and highly effective fungicide combinations, offering valuable options for integrated *Alternaria* blight management in canola.

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Introduction

Canola (*Brassica napus*), also known as oilseed rape or rapeseed, is an important oilseed crop cultivated mainly in temperate and subtropical regions for edible oil production (Neik et al., 2020). In Pakistan, *B. napus* is considered a vital oilseed crop, contributing nearly 20% of the country's edible oil requirements (Subhani et al., 2018).

The yield potential of canola is strongly influenced by various abiotic and biotic factors. Achieving optimum yield, which is the ultimate goal of all crop management practices, including fungicide and pesticide application, as well as agronomic interventions, remains a significant challenge (Zhang, and Flottmann, 2016). Among biotic stresses, pathogens such as bacteria, viruses, and fungi cause substantial yield losses in canola.

Several fungal diseases are particularly destructive to canola production. These include blackleg caused by *Leptosphaeria maculans*, Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, clubroot caused by *Plasmodiophora brassicae*, damping-off caused by *Rhizoctonia solani*, downy mildew caused by *Peronospora parasitica*, white leaf spot caused by *Mycosphaerella capsellae*, white rust (staghead) caused by *Albugo candida*, and *Alternaria* blight caused by *Alternaria brassicae* (Van de Wouw et al., 2016). Among these, *Alternaria* blight is regarded as the most prevalent and devastating fungal disease of canola worldwide (Jat et al., 2019; Horn and Shimelis, 2020).

Yield losses caused by *Alternaria* blight are considerable, with an average reduction of 36.88% in seed production (Kumar et al., 2015). After soybean, canola is the second most important oilseed crop globally; however, *Alternaria* blight poses a major constraint, with reported yield losses reaching up to 70%. At present, this disease is responsible for nearly 50% yield losses in canola production worldwide (Pandey et al., 2023).

In addition to pathogens, the use of pesticides also influences crop health and quality. Antioxidant molecules such as ascorbic acid, α-tocopherol, threonic acid, 4-hydroxybutyric acid, folic acid, and total phenolic compounds are reduced by 29-85% following pesticide applications containing copper hydroxide [$Cu(OH)_2$] (Zhao et al., 2017). Although pesticides can provide effective protection against pests, they may also exert stress on non-target organisms, thereby posing a threat to ecosystem stability (Bourguet, and Guillemaud, 2020). Recent advances in pesticide formulations highlight the use of novel materials as efficient delivery agents for insecticides, herbicides, fungicides, defoliants, and biocides. These next-generation pesticides improve the bioavailability of active ingredients through controlled release and targeted delivery, thereby enhancing efficacy while reducing environmental risks (Kah and Hofmann, 2014).

In this context, the present study was designed with two main objectives: first, to evaluate the varietal response of canola germplasm against *A. brassicae*, and second, to conduct *in vivo* evaluation of fungicides for the effective management of *Alternaria* blight in canola.

Materials and Methods

Procurement of germplasm

The germplasm of the *B. napus* varieties used in this study was obtained from the Oilseed Research Institute, Ayyub Agriculture Research Institute (AARI), Faisalabad, Pakistan. During the growing season, all recommended agronomic practices were followed. The varieties used for evaluation against *Alternaria* leaf blight are listed in Table 1.

Table 1. Canola varieties used in the study.

Sr. No.	Name of Variety	Year of Release	Source
1	Super Canola	2018	AARI, FSD
2	Super Raya	2016	AARI, FSD
3	Rohi Sarsoon	2016	AARI, FSD
4	AARI Canola	2016	AARI, FSD
5	Faisal Canola	2011	UAF, FSD
6	Punjab Canola	2009	AARI, FSD
7	Khanpur Raya	2021	AARI, FSD
8	Anmol Raya	1988	AARI, FSD

Screening of germplasm

The collected *B. napus* germplasm was evaluated for resistance against *A. brassicae* under field conditions. Disease severity was recorded at regular intervals following symptom appearance. A 0-7 rating scale was used, where 0 indicated healthy plants with no visible symptoms and 7 corresponded to plants showing complete (100%) disease severity (Table 2).

Disease incidence (%) was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseasedplants observed}}{\text{Total number of plants observed}} \times 100$$

The severity scores and incidence values were used to classify the germplasm into different resistance categories, ranging from resistant to highly susceptible. Observations were recorded on a plant basis, and mean values were used for subsequent statistical analysis.

Table 2. Disease severity assessment scale (%).

Disease Severity scale	Percentage (Leaf area covered by pustules)
0	No symptoms
1	1-10%
2	11-20%
3	21-30%
4	31-50%
5	51-75%
6	>75%
7	100

In vivo evaluation of fungicides against *A. brassicae*

The efficacy of six fungicides (Table 3) was evaluated against *Alternaria brassicae* under in vivo conditions.

Table 3. Chemicals used in the experiment.

Sr. No.	Name of chemicals
1	Control
2	Mancozeb
3	Carbendazim
4	Metalaxly
5	Mancozeb + Carbendazim
6	Mancozeb + Metalaxly
7	Mancozeb + Carbendazim + Metalaxly

Artificial inoculation was carried out at the four-leaf stage by spraying a conidial suspension of *A. brassicae* (1×10^5 CFU ml^{-1}) onto canola plants. The experiment consisted of eight plots, one for each variety. Each plot comprised five rows, with one row considered as one replication, containing 10 plants per row. Thus, each variety had five replications. Treatments were arranged in a Completely Randomized Block Design (CRBD), with each treatment repeated four times.

Two foliar applications of fungicides were made at 15-day intervals. Ten days after the second application, disease severity (%) was recorded following the 0-7 severity scale (Table 2) (Aneja and Agnihotri, 2016). All treatments were compared with the untreated control to evaluate fungicidal efficacy.

Environmental data collection

Meteorological data were obtained from the Agromet Department, Regional Agriculture Research Institute (RARI), Bahawalpur, Punjab, Pakistan, for a five-year period (2018-2023). The recorded variables included maximum and minimum temperatures, relative humidity, rainfall, and wind speed during the months of February and March, corresponding to the critical period for canola blight development.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) following the procedures of Steel et al. (1997) using CRBD to determine significant differences among treatments. Principal component analysis (PCA) was also performed to identify patterns of variation and to distinguish the most resistant genotypes.

Results

Response of canola genotypes to Alternaria blight under field conditions

Field screening of canola genotypes against *Alternaria* blight revealed clear differences in disease reaction (Table 4). None of the tested genotypes exhibited complete immunity, high resistance, or resistance to the disease. Four genotypes, namely Anmol Raya, Khanpur Raya, Super Raya, and AARI Canola, were categorized as moderately resistant, indicating their comparatively better performance under natural field conditions.

Conversely, a large proportion of the genotypes displayed higher disease susceptibility. Rohi-Sarsoon, Punjab Canola, Faisal Canola, Peela Raya, and Super Canola were classified as highly susceptible, showing severe disease symptoms. No genotype fell into the susceptible or moderately susceptible categories.

Overall, the results indicate the absence of immune or resistant genotypes in the tested panel, with only a few lines showing moderate resistance, while most commercial cultivars were highly susceptible to *Alternaria* blight.

Table 4. Field screening of canola genotypes against Alternaria blight.

Rating scale %	Disease Reaction	Lines/Genotypes
1	Immune	-
2	Highly Resistant	-
3	Resistant	-
4	Moderately Resistant	Anmol Raya, Khanpur Raya, Super Raya, AARI Canola
5	Highly Susceptible	Rohi-Sarsoon, Punjab Canola, Faisal Canola, Peela Raya, Super Canola
6	Susceptible	-
7	Moderately Susceptible	-

Disease incidence and severity patterns among canola genotypes

The mean disease severity among canola genotypes ranged from 40.33% to 56.00%. The highest disease severity was observed in Rohi-Sarsoon (56.00%), closely followed by Punjab Canola (55.00%). In contrast, Super Raya (41.00%) and AARI Canola (40.33%) exhibited the lowest severity values, indicating that these two genotypes were the most tolerant among the tested germplasm (Figure 1).

The mean disease severity also ranged from 40.33% to 56.00%, while disease incidence varied between 45.67% and 82.33%. Faisal Canola recorded the highest values for both disease incidence (82.33%) and severity (52.33%), followed by Punjab Canola (73.66% and 55.00%, respectively). Conversely, AARI Canola (45.67%, 40.33%) and Super Raya (46.66%, 41.00%) demonstrated the lowest values, confirming their relative tolerance to *Alternaria* blight under field conditions (Figure 1).

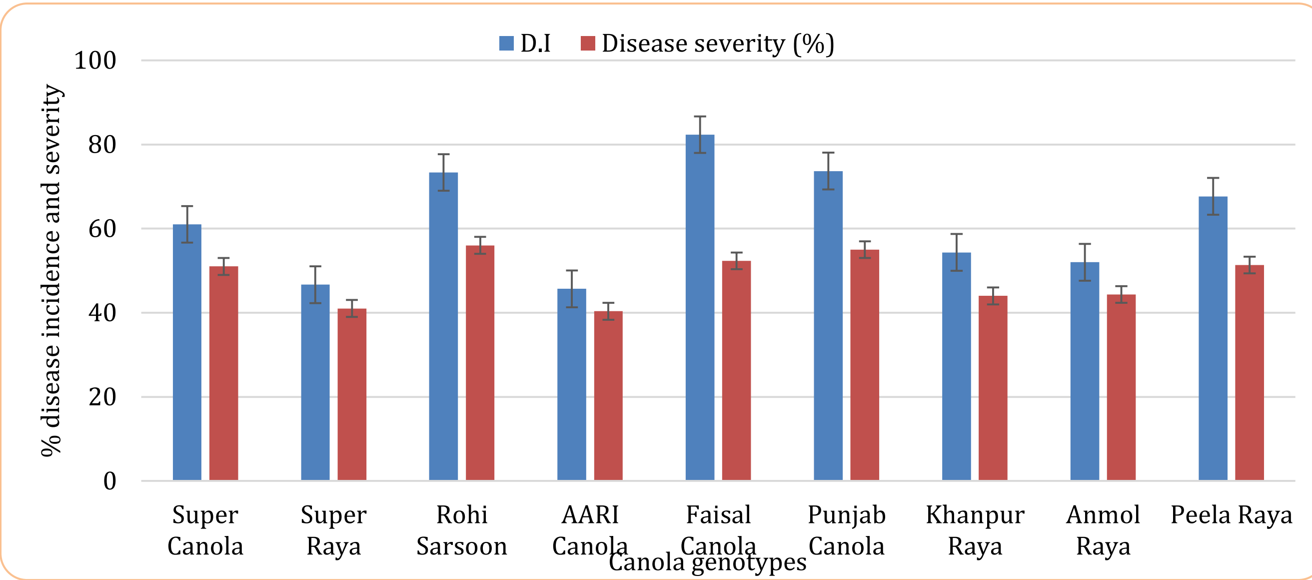

Figure 1. Relationship between disease incidence and severity in canola genotypes against *Alternaria* blight before fungicide application.

Efficacy of fungicides against *Alternaria* blight in selected canola varieties

Pre-treatment disease incidence and severity in selected canola varieties

Both varieties, Rohi Sarsoon and Punjab Canola, exhibited high disease incidence (~73%) and disease severity (>55%) before fungicidal application, indicating high susceptibility to the pathogen at the initial screening stage (Figure 2).

Comparison of fungicide efficacy on selected canola genotypes after 7 days

After 7 days, all fungicide treatments reduced disease severity index (DSI) compared to the control, with varying efficacy. The combinations mancozeb + carbendazim + Metaxyl and mancozeb + Metaxyl showed the lowest DSI in both Rohi Sarson and Punjab Canola, indicating superior performance. Punjab Canola exhibited slightly lower DSI than Rohi Sarson, suggesting greater tolerance. Metaxyl and mancozeb alone had moderate effects, while carbendazim alone was least effective. The untreated control recorded the highest DSI, highlighting the importance of fungicidal application (Figure 3).

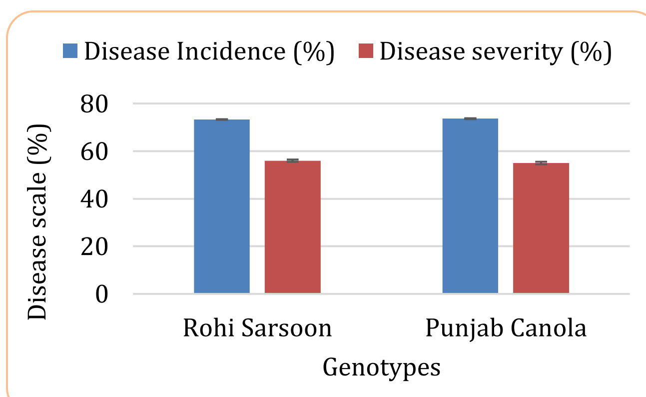

Figure 2. Disease incidence and severity of selected lines before spray.

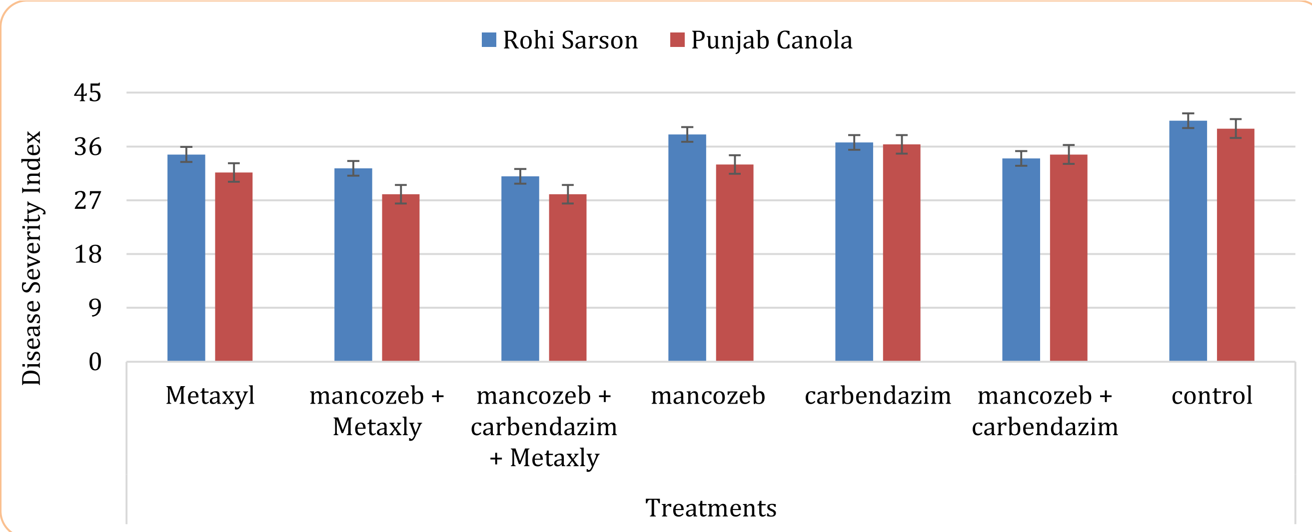

Figure 3. Comparison of fungicide efficacy on canola genotypes after 7 days.

Fungicidal efficacy against Alternaria blight in canola after 14 days

The two canola genotypes (Rohi Sarson and Punjab Canola) responded differently to fungicidal treatments after 14 days. The combination of mancozeb + carbendazim + Metaxyl was most effective, reducing DSI to 24.00 in Punjab Canola and 29.67 in Rohi Sarson. Mancozeb + Metaxyl also performed well (24.67 and 28.67, respectively). In contrast, single fungicides, mancozeb, carbendazim, and Metaxyl, showed higher DSI values, while the untreated control recorded the highest severity (42.00 and 41.33) (Figure 4). Overall, combination treatments, particularly mancozeb + carbendazim + Metaxyl, provided superior protection, with Punjab Canola showing lower disease severity than Rohi Sarson.

Comparative Efficacy of Fungicides on Canola Genotypes after 21 Days

Figure 5 shows that all fungicidal treatments significantly reduced disease severity compared to the control in both Punjab Canola and Rohi Sarsoon. Among the treatments, Mancozeb + Metalaxyl recorded the lowest severity (22% and 20%, respectively), followed closely by Metalaxyl alone (26% and 21.33%). The combination of three fungicides (Mancozeb + Carbendazim + Metalaxyl) and Carbendazim alone also performed well, whereas Mancozeb alone was relatively less effective but still superior to the untreated control. Overall, Mancozeb + Metalaxyl proved the most effective across both genotypes after 21 days.

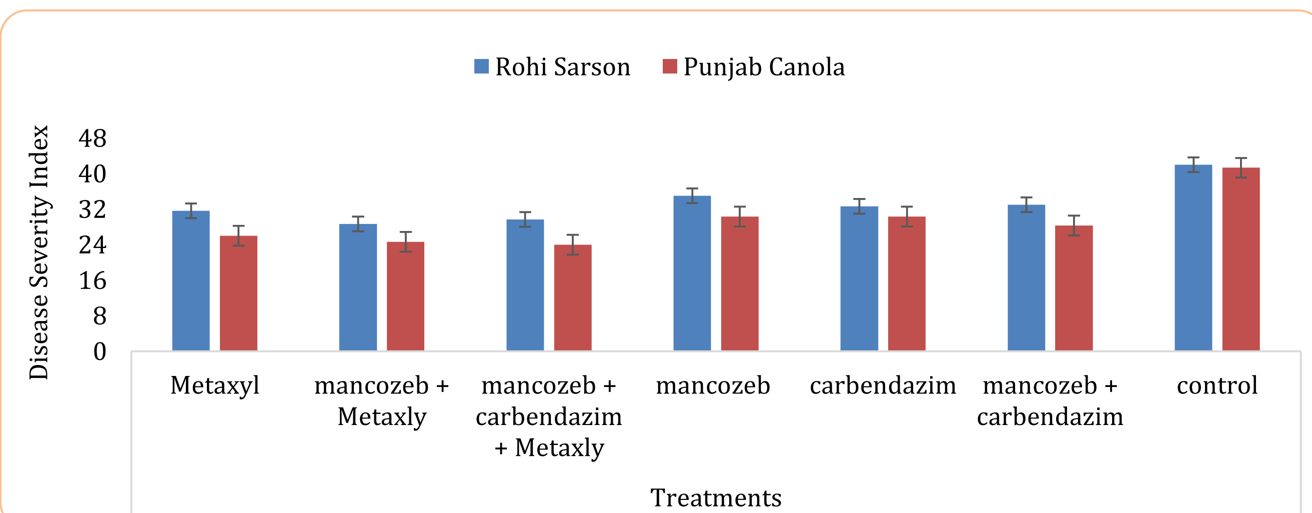

Figure 4. Comparison of fungicide efficacy in selected canola genotypes after 14 days.

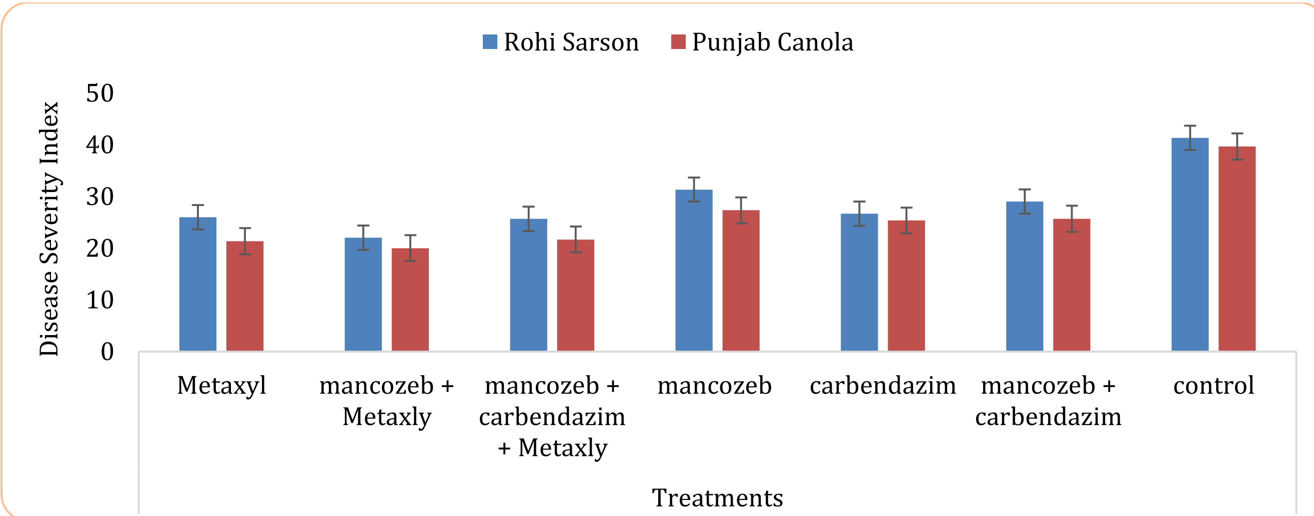

Figure 5. Comparison of fungicide efficacy on selected canola genotypes after 21 days.

Discussion

Among the evaluated genotypes, Anmol Raya, Khanpur Raya, Super Raya, and AARI Canola exhibited relatively higher levels of tolerance, with disease severity remaining below 45%. These results are consistent with the findings of Sharma et al. (2002), who also classified these lines as moderately resistant. The underlying resistance mechanisms, however, remain unclear and warrant further investigation through genetic and biochemical studies.

In contrast, Rohi-Sarsoon and Punjab Canola were highly susceptible, showing mean disease severity exceeding 55%. This observation is in agreement with earlier reports identifying these cultivars as highly prone to *Alternaria* blight (Meena et al., 2010). Similarly, disease incidence followed the same pattern: Faisal Canola recorded the highest incidence (82.33%) compared to only 45-47% in tolerant genotypes. These findings validate the differential responses among genotypes and align with the report of Kolte et al. (1987), who noted that disease incidence in susceptible cultivars can be nearly double that of resistant ones under field conditions.

Overall, the screening confirmed significant genetic variability for *Alternaria* blight resistance within *B. napus* germplasm. The tolerant genotypes identified here may serve as potential sources of resistance for breeding programs aimed at developing cultivars with durable resistance. Further studies on the inheritance of resistance factors would also facilitate the application of marker-assisted selection in resistance breeding.

Fungicide evaluations on highly susceptible genotypes provided a reliable means to assess the protective efficacy of chemical treatments under high disease pressure, minimizing confounding effects of genetic resistance. The results can be extrapolated to improve management strategies for inherently susceptible commercial cultivars such as Rohi-Sarsoon and Punjab Canola, which dominate canola production in India (Meena et al., 2017).

The complementary actions of protectant, systemic, and targeted fungicides likely contributed to sustained control up to 21 days after application. Mancozeb, acting as a surface protectant, provided baseline coverage; Carbendazim inhibited spore germination; and Metalaxyl targeted Oomycetes (Kumar et al., 2015). Nevertheless, disease suppression declined for all treatments at 21 days compared to 14 days, reflecting reduced residual activity. This trend is consistent with earlier observations that fungicide efficacy against *Alternaria* blight diminishes beyond 15-21 days (Sharma and Kolte, 1994; Gullino et al., 2010), likely due to weathering and possible pathogen adaptation.

At 21 days, individual fungicides provided relatively low protection: Mancozeb (40.5%), Carbendazim (29.33%), and Metalaxyl (27.33%). Mancozeb outperformed the systemic fungicides, likely because of its multi-site mode of action, which reduces the risk of resistance buildup.

Beyond fungicides, integrated strategies should be emphasized. Synthetic fertilizers and pesticides have improved crop yields globally (Schreinemachers, and Tipraqsa, 2012; Lamberth et al., 2013), but challenges remain in achieving optimal spray deposition, targeted delivery, and retention on leaf surfaces (Dong et al., 2015; Bueno et al., 2017; Mahmud et al., 2017). Biocontrol agents such as *Bacillus* spp. have shown promise against fungal pathogens (Fatima et al., 2023; Maalik et al., 2023; Malik et al., 2024), offering potential eco-friendly alternatives. Although fungicides remain the primary option for managing canola white rust, irregular application patterns and insufficient field surveillance in India (IASRI, 2006; Meena, 2015; Mehta, 2021) highlight the need for more consistent and informed disease management strategies.

Conclusion

Canola is a vital oilseed crop in Pakistan but remains vulnerable to *Alternaria* blight, a major yield-reducing disease. Screening of nine genotypes revealed substantial variability in disease response: five genotypes were susceptible, whereas four exhibited moderate resistance. Fungicide assessments on the two most susceptible cultivars, Rohi-Sarsoon and Punjab Canola, demonstrated that Mancozeb + Metalaxyl + Carbendazim and Mancozeb + Metalaxyl combinations provided the most effective control, significantly reducing disease severity compared to untreated controls.

These findings emphasize the dual importance of (i) identifying resistant genotypes as potential breeding resources, and (ii) optimizing fungicide strategies for immediate disease management. Together, these approaches can strengthen integrated disease management programs and contribute to more sustainable canola production.

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Authors' Contributions

MSIM, TM and MR designed the study; MSA, SA, MRB prepared the materials, collected and analyzed the data; HMA helped in disease scoring; MR, TM and AM supervised the studies; AAZ, AQ, and TM wrote the manuscript; All the authors proofread and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

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