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IN VITRO AND IN VIVO EFFICACY OF DIFFERENT FUNGICIDES AGAINST ALTERNARIA LEAF SPOT OF CABBAGE CAUSED BY *ALTERNARIA BRASSICICOLA*.

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ABSTRACT

The effectiveness of different fungicides under both *in vitro* and field conditions against *Alternaria brassicicola* was assessed during 2022–23 at the College of Horticulture, Bengaluru. Copper hydroxide (86.85%) emerged as the most effective contact fungicide, outperforming mancozeb (70.97%) and chlorothalonil (38.19%). Among systemic fungicides, Tebuconazole 25% EC achieved 100% inhibition at all tested concentrations, followed by Propiconazole 25% EC (96.66%). The combination fungicide Fluxapyroxad 250 + Pyraclostrobin 250 SC exhibited 93.65% inhibition, whereas Carbendazim 50% WP was the least effective (39.29%). Field evaluations conducted during the 2024 *Kharif* season tested seven fungicides, with all treatments significantly reducing disease severity compared to the untreated control (PDI 36.48%). Tebuconazole 25% EC at 0.05% was the most effective, recording the lowest PDI (6.66%), followed by Trifloxystrobin 25% + Tebuconazole 50% WG (7.66%), Fluxapyroxad 250 + Pyraclostrobin 250 SC (11.04%), Propiconazole 25% EC (11.98%), and Hexaconazole 5% EC (11.99%). Contact fungicides Mancozeb 75% WP (29.93%) and Copper Hydroxide 53.8% DF (26.40%) were the least effective under field conditions. These findings highlight the superior efficacy of Tebuconazole 25% EC and combination fungicides in managing *Alternaria brassicicola* both *in vitro* and *in vivo*.

Key words: Alternaria leaf spot, *Alternaria brassicicola*, Fungicides

Introduction

Cabbage (*Brassica oleraceae* var. *capitata* L.) is a leafy head vegetable belonging to *Brassicaceae* family. It is grouped as cole crops, evolved from a single wild species *Brassica oleracea* var. *oleracea* (*sylvestris* L.), commonly known as wild cabbage or ‘Colewort’ (Glory *et al.*, 2022). Cabbages are utilized in various manners for eating *viz.*, for salads, boiling, pickling and sauerkraut. Health benefits include reduction of some forms of cancers, improving digestion and useful for managing diabetes. Nutritionally, they are high in α carotene, vitamin A, C, K and fibre. It is a rich source of protein (1.4 %), minerals such as calcium (0.73 mg/100 g of edible portion),

phosphorus (0.38 %), potassium (2.71 %), sulphur (1.16 %), iron (205 ppm) and every 100 g of edible portion contain vitamins like A (400 mg), B1 (27 mg) and C (100 mg) (Tu. 2015).

According to the Food and Agriculture Organization (FAO) of the United Nations the global area harvested under cabbage is 2,326,179 ha, with a production of 72.60 MT. The top countries in both area and production are China and India in 1st and 2nd position accounting for the 43 per cent (10.05 lakh ha) and 18 per cent (4.23 lakh ha) of global area respectively. In 2022, The cumulative production of cabbage in China is around 356.04 lakh MT which is 49 per cent share of global production and

Table 1: Disease scale (0-5) for scoring *Alternaria* leaf spot.

Scale	Description
0	Leaves remained symptom-free
1	Small, uneven brown lesions covering up to 1% of the leaf area.
2	Small, uneven brown lesions with concentric rings occupying 1–10% of the leaf area
3	Enlarging, irregular brown lesions with concentric rings occupying 11–25% of the leaf area
4	Merging lesions develop into irregular brown areas with concentric rings, covering 26–50% of the leaf surface. The stalk and petioles also exhibit similar symptoms
5	Lesions coalesce to form irregular, dark brown areas with concentric rings, covering more than 51% of the leaf surface. Similar lesions are also observed on the stalks and petioles.

coming to India total production for the calendar year 2022 is 98.3 lakh MT with 13 per cent share in global production (Anon. 2022). Multiple states across India, including Bihar, Assam, Odisha, Uttar Pradesh, West Bengal, Maharashtra and Karnataka, are leading producers of cabbage. The total cabbage production in Karnataka for the recent year (2022-23) was reported to be 509.71 MT with area of 11.05 thousand hectares. This production is part of the state's broader horticultural output, with this Karnataka being a significant contributor to India's overall vegetable production. (Anon. 2023)

Cabbage faces various challenges, both biological and environmental. Major diseases in cabbage are Black rot, Downy mildew, Wire stem, Leaf spot and blight, Yellows or Fusarium Wilt, Black leg and Clubroot of Cabbage. Among these diseases, *Alternaria* leaf spot stands out as a prevalent fungal disease. This particular disease is primarily incited by two species of *Alternaria*: *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schweintiz) Wiltshire in cabbage (Humpherson-Jones. 1993). *Alternaria* leaf spot is challenging to manage due to the pathogen's ability to survive in infected seeds, plant debris in the soil, and on collateral hosts like nearby cruciferous crops or weeds. The use of fungicides remains one of the oldest and most effective methods for managing this disease. Different fungicides are commercially available, and many others are undergoing evaluation in various research laboratories.

Considering this the present study was conducted for the evaluation of various fungicides for their *in vitro* and *in vivo* effectiveness against *Alternaria* leaf spot in cabbage.

Table 2: Treatment details of field evaluation of fungicides against *Alternaria* leaf spot disease in cabbage.

Sl. No.	Treatments	Concentration (%)
1	Mancozeb 75% WP	0.25
2	Copper hydroxide 53.8% DF	0.25
3	Propiconazole 25% EC	0.10
4	Hexaconazol 5% SC	0.10
5	Tebuconazole 25% EC	0.10
6	Tebuconazole 50 + Trifloxystrobin 25% WG	0.07
7	Fluxapyroxad 250 + pyraclostrobin 250 SC	0.05
8	Untreated control	—

Material and Methods

Assessment of fungicides for their *in vitro* efficacy against *Alternaria brassicicola*

The present study was conducted in the Department of Plant Pathology, College of Horticulture, Bengaluru. The effectiveness of three contact, five systemic and two combination fungicides at different concentrations were assayed against *A. brassicicola* in the laboratory conditions by "Poison food technique" (Grover and Moore, 1962) on Potato Dextrose Agar medium. Each conical flask containing 100 ml of potato dextrose agar medium was prepared and autoclaved at 121°C and 15lbs pressure for 20 min. Fungicide suspensions were prepared by adding the appropriate amount of fungicide to molten PDA to achieve the desired concentration based on the active ingredient. 15 ml of the poisoned medium were poured into each sterilized petri plate, with controls maintained without fungicide. A 5 mm disc of ten-day-old *Alternaria brassicicola* was positioned at the center of the poisoned medium and incubated at $27 \pm 1^\circ\text{C}$ for seven days. Each treatment was replicated three times.

The percentage of mycelial growth inhibition compared to the control was calculated using the formula of Vincent (1947) as follows:

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

Where,

I - Per cent growth inhibition of mycelium

C - Growth of mycelium in control

T - Growth of mycelium in treatment

Field evaluation of fungicides against of *A. brassicicola*

The field experiments were conducted to manage *Alternaria* leaf spot disease in cabbage during *Kharif* 2024 at Regional Horticulture Research and Extension centre (RHREC), Bengaluru. The experiments were

Table 3: *In vitro* evaluation of contact fungicides against *Alternaria brassicicola*.

S. No.	Fungicides	Per cent inhibition of mycelial growth			Mean
		Concentration (%)			
		0.15	0.20	0.25	
1	Mancozeb 75 % WP	65.5 (54.03) *	71.57(57.77)	75.86(60.16)	70.97(57.32)
2	Copper hydroxide 53.8 % DF	83.32(65.81)	86.57(68.50)	90.68(72.19)	86.85(68.84)
3	Chlorothalonil 75 %WP	30.37(33.43)	40.64(39.60)	43.58(41.30)	38.19(38.11)
	Mean	59.73(51.09)	66.26(55.29)	70.04(57.89)	
Fungicides		Concentration		Fungicides × Concentration	
S.Em. ±		0.18		0.31	
CD @ 1%		0.73		1.27	

designed with seven fungicides and three replications, arranged in a Randomized Block Design (RBD) with plots measuring 4.5m × 3.6m where each plot consist of 28 plants. the first spray was applied when the initial symptom was observed and two sequential sprays at the interval of 10 days were given. The observations were recorded before fungicide application, after 10th day of first, second and third application of fungicide spray. 10 plants were randomly chosen in each treatment, and the disease severity on the foliage was assessed using a 0-5 scale (Mayee and Datar, 1986).

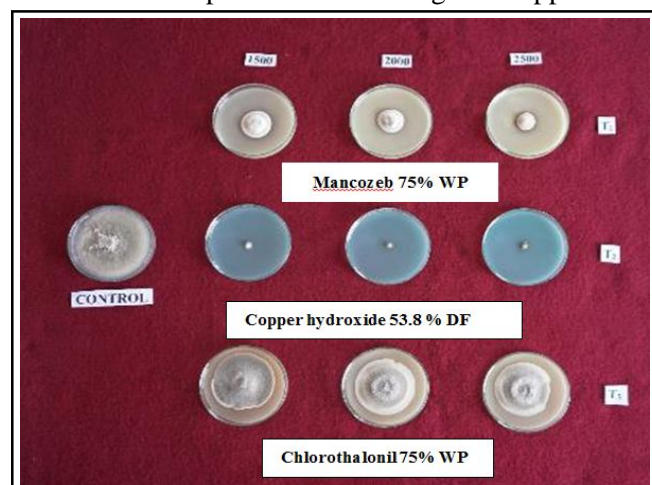
Per cent Disease Index (PDI) was worked out using the following formula (Wheeler, 1969).

$$PDI = \frac{\text{Sum of the individual disease ratings}}{\text{Number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

Results and Discussion

Assessment of fungicides for their *in vitro* efficacy against *Alternaria brassicicola* causing *Alternaria* leaf spot of cabbage

Alternaria leaf spot is challenging to manage due to the pathogen's ability to survive in infected seeds, plant debris in the soil, and on collateral hosts like nearby cruciferous crops or weeds. Fungicide application

**Fig. 1:** *In vitro* evaluation of contact fungicides against *Alternaria brassicicola*.

remains a conventional and reliable method for disease control. A variety of fungicides are commercially available, and many others are undergoing evaluation in various research laboratories. Evaluating the efficacy of available chemical fungicides under *in vitro* conditions is crucial for designing an effective spray schedule for field application.

The results indicate a significant variation within the group of contact fungicides in their ability to inhibit the radial growth of *A. brassicicola*. Among the contact fungicides tested, Copper hydroxide 53.8% DF demonstrated the highest mean mycelial inhibition at 86.85 per cent, outperforming the other two fungicides in terms of efficacy. Mancozeb 75 % WP showed 70.97% inhibition, while the lowest inhibition rate of 38.19 per cent was observed with Chlorothalonil 75 %WP. The findings also showed that the mycelial growth inhibition percentage increased with higher fungicide concentrations. At a concentration of 0.25 per cent,

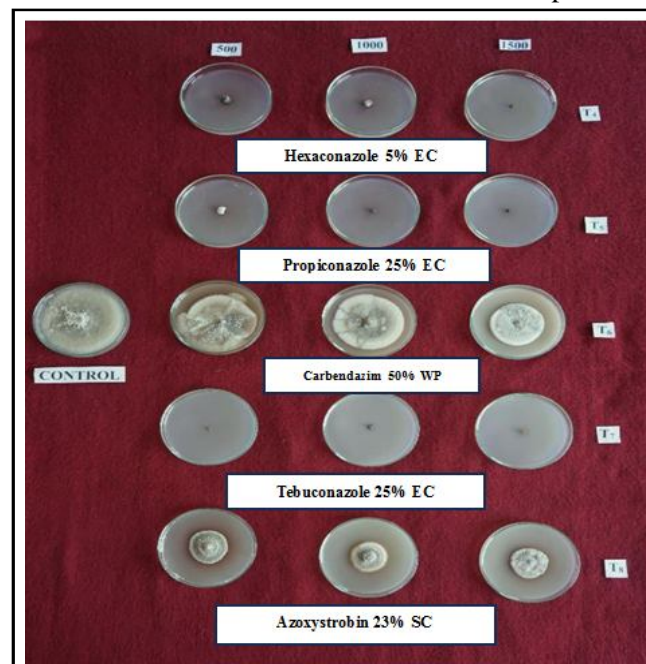
**Fig. 2:** *In vitro* evaluation of systemic fungicides against *Alternaria brassicicola*.

Table 4: *In vitro* evaluation of systemic and combination fungicides against *Alternaria brassicicola*.

S. No.	Fungicides	Per cent inhibition of mycelial growth			Mean
		Concentration (%)			
		0.05	0.10	0.15	
1	Hexaconazole 5 % EC	89.79 (71.37) *	90.82(72.36)	100(89.63)	93.53(77.79)
2	Propiconazole 25 % EC	90(71.56)	100(89.63)	100(89.63)	96.66(83.61)
3	Carbendazim 50 % WP	24.62(29.74)	42.44(40.64)	50.83(45.47)	39.29(38.62)
4	Tebuconazole 25 % EC	100(89.63)	100(89.63)	100(89.63)	100(52.03)
5	Azoxystrobin 23 % SC	58.86(50.10)	63.47(52.81)	64.12(53.20)	62.15(89.63)
6	Trifloxystrobin 25 + Tebuconazole 50 % WG	81.58(64.58)	85.49(67.61)	86.61(68.54)	84.56(66.91)
7	Fluxapyroxad 250 + Pyraclostrobin 250 SC	92.96(74.62)	93.67(75.44)	94.34(76.26)	93.65(75.44)
	Mean	76.83(64.51)	82.27(69.73)	85.12(73.20)	
Fungicides		Concentration		Fungicides × Concentration	
S. Em. ±		0.15		0.39	
CD @ 1%		0.57		1.50	

Copper hydroxide 53.8% DF achieved the highest inhibition of 90.68 per cent, followed by Mancozeb 75% WP at 75.86 per cent, while Chlorothalonil 75% WP recorded the lowest inhibition at 70.04 per cent.

Out of the five systemic fungicides evaluated against *A. brassicicola*, Tebuconazole 25 % EC (0.05 %, 0.10%, and 0.15%) achieved complete mycelial growth inhibition (100%), followed by Propiconazole 25 % EC, which showed 96.66 per cent inhibition. Of the two combination fungicides evaluated, Fluxapyroxad 250 + Pyraclostrobin 250 SC exhibited the highest inhibition of mycelial growth in the test fungi (93.65 %). Among all the fungicides tested, Tebuconazole 25% EC demonstrated the highest average mycelial inhibition of *A. brassicicola*, achieving 100 per cent inhibition, followed by Propiconazole 25 % EC at 96.66 per cent, while Chlorothalonil 75 % WP had the lowest at 38.19 per cent. Increased fungicide concentrations generally led to higher inhibition rates.

The effectiveness of seven fungicides was evaluated in field trials during the *Kharif* season of 2024 for *Alternaria* leaf spot disease management in cabbage. The results demonstrated that all fungicides significantly reduced the disease severity compared to the untreated control, though the degree of reduction varied between treatments. 10 days after the third application, the percent disease index (PDI) of *Alternaria* leaf spot disease in cabbage ranged from 6.66 per cent to 29.93 per cent, compared to 36.48 per cent in the untreated control.

Among the fungicides, Tebuconazole 25% EC at 0.10 per cent concentration exhibited the most effective control, achieving the lowest PDI of 6.66 per cent, demonstrating clear superiority over all other treatments. Trifloxystrobin 25 % + Tebuconazole 50 % WG at 0.07 per cent followed closely, with a PDI of 7.66 per cent. Other effective treatments included Fluxapyroxad 250 +

Pyraclostrobin 250 SC, which recorded a PDI of 11.04 per cent at 0.05 per cent concentration. While, Propiconazole 25 % EC at 0.10 per cent and Hexaconazole 5% EC at 0.10 per cent, both of which resulted in almost identical PDI of 11.98 per cent and 11.99 per cent, respectively. This suggests that although all treatments effectively reduced disease severity, Tebuconazole 25% EC was the most successful in managing *Alternaria* leaf spot disease under field conditions.

The treatment that showed the highest percent disease index (PDI) was observed in Mancozeb 75% WP at a concentration of 0.25 per cent, with a PDI reaching 29.93 per cent. This result indicates that Mancozeb was the least effective fungicide among those evaluated, offering limited control over the disease. Following Mancozeb, Copper hydroxide 53.8% DF recorded the next highest PDI of 26.40 per cent, further suggesting reduced efficacy in managing the disease.

Among the different treatments tested, the highest head yield of 58 t ha⁻¹ was achieved with Trifloxystrobin 25 % + Tebuconazole 50 % WG applied at 0.07 per cent concentration. This treatment demonstrated better

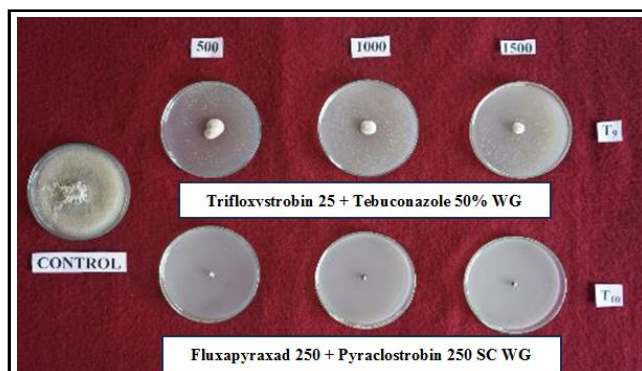
**Fig. 3:** *In vitro* evaluation of combi fungicides against *Alternaria brassicicola*.

Table 5: Field evaluation of different fungicides for the management of Alternaria leaf spot of cabbage during *Kharif* 2024.

Treatment		C (%)	Pre-treatment	Per cent disease index 10 days after			Yield (Kg/plot)	Yield (t/ha)	B:C
				1 st spray	2 nd spray	3 rd spray			
T1	Mancozeb 75% WP	0.25	19.71 (26.35) *	24.02(29.34)	28.14(32.03)	29.93(33.16)	48	30.47	1.21
T2	Copper hydroxide 53.8% DF	0.25	17.34(24.60)	22.01(27.97)	25.04(30.02)	26.40(30.91)	46	29.20	1.13
T3	Propiconazole 25% EC	0.10	19.56(25.57)	16.84(22.75)	14.59(19.02)	11.98(14.95)	52	33.01	1.44
T4	Hexaconazole 5% EC	0.10	20(26.24)	18.03(24.22)	14.47(22.45)	11.99(20.24)	53	33.65	1.49
T5	Tebuconazole 25% EC	0.10	18.63(26.56)	14.98(25.12)	10.63(22.35)	6.66(20.25)	56	35.55	1.63
T6	Trifloxystrobin 25 + Tebuconazole 50% WG	0.07	21.5(26.39)	15.86(23.85)	10.56(18.96)	7.66(16.05)	58	36.82	1.71
T7	Fluxapyraxad 250 + Pyraclostrobin 250 SC	0.05	18.27(25.41)	15.09(22.85)	13.05(21.17)	11.04(19.40)	53	33.65	1.46
T8	Untreated control	—	18.49(25.56)	26.87(31.22)	32.70(34.87)	36.48(37.15)	42	26.66	0.98
CD @ 5%			NS	1.17	0.87	1.02	2.03	1.29	
S.Em. ±				0.40	0.29	0.35	0.69	0.44	
CV%				5.41	4.15	5.13	3.52	3.52	
C: Concentration (%)									

effectiveness compared to others, highlighting its potential efficacy in boosting head yield. The second highest yield was recorded with Tebuconazole 25% EC (0.10%), resulting in 56 t ha⁻¹. In contrast, the control group, which received no fungicidal treatment, recorded the lowest head yield at 42 t ha⁻¹.

Discussion

In an *in vitro* evaluation of fungicides against *Alternaria brassicicola*, copper hydroxide (86.85%) was the most effective contact fungicide, outperforming mancozeb (70.97%) and chlorothalonil (38.19%). Among systemic fungicides, Tebuconazole 25% EC achieved 100 per cent inhibition at all tested concentrations, followed by Propiconazole 25 % EC (96.66%). The combination of Fluxapyrad 250 + Pyraclostrobin 250 SC showed 93.65 per cent inhibition, while among all fungicides Carbendazim 50 % WP was least effective (39.29%). The results are in agreement with results of Kiran *et al.*, 2018 who reported the efficiency of Tebuconazole 25% EC at 0.05 per cent in mycelial growth inhibition of test fungi. Tu and Somasekhara (2015) reported the efficacy of fungicides against *A. brassicicola* on cabbage under laboratory conditions and at a concentration of 1000 ppm, all the evaluated fungicides completely inhibited fungal growth, with Tebuconazole 25 % EC and Propiconazole 25 % EC achieving 100 per cent suppression. Das *et al.*, (2023) found that inhibition per cent at 0.20 per cent of mancozeb against *A. brassicicola* were consistent with our findings.

During the 2024 *Kharif* season, seven fungicides were tested in field conditions for managing Alternaria leaf spot in cabbage. All treatments significantly reduced

disease severity compared to the control (PDI 36.48%), with PDI values ranging from 6.66 per cent to 29.93 per cent ten days after the third spray. Tebuconazole 25% EC at 0.05 per cent was the most effective, recording the lowest PDI (6.66%), followed by Trifloxystrobin 25 % + Tebuconazole 50 % WG (7.66%), Fluxapyrad 250 + Pyraclostrobin 250 SC (11.04%), Propiconazole 25 % EC (11.98%), and Hexaconazole 5% EC (11.99%).

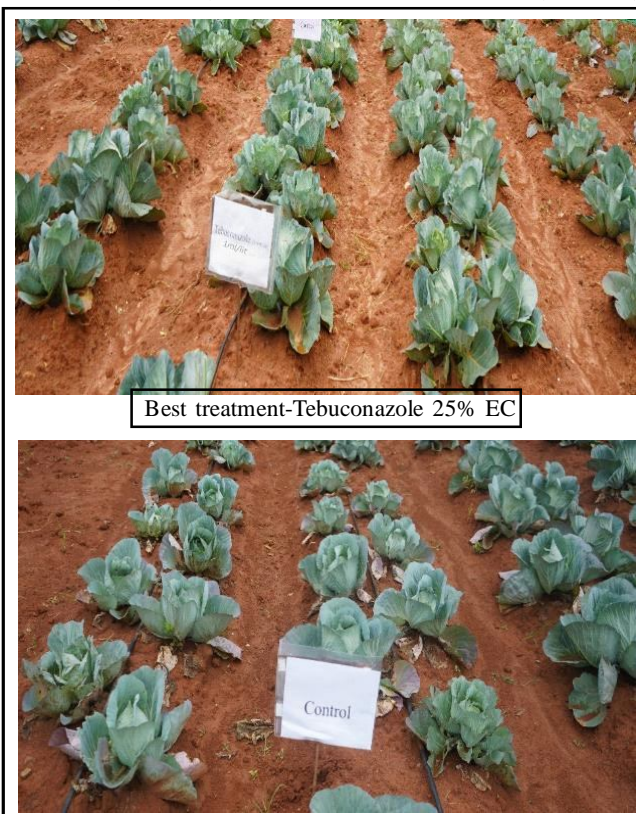


Fig. 4: Effect of different fungicides in field evaluations against Alternaria leaf spot of cabbage.

Mancozeb 75 % WP (29.93 %) and Copper Hydroxide 53.8 % DF (26.40 %) were the least effective among the tested fungicides. Trifloxystrobin 25% + Tebuconazole 50% WG (0.07%) produced the highest cabbage yield (58 t/ha), followed by Tebuconazole 25% EC (0.10%) at 56 t/ha, while the control plot had the lowest yield (42 t/ha). The field results are in agreement with the results of Tu and Somasekhara (2015) where they found that among the eleven fungicides used for controlling *Alternaria* leaf spot disease in cabbage. Tebuconazole 25 % EC was most effective in reducing the per cent disease index followed by Trifloxystrobin 25 % + Tebuconazole 50 % WG and Propiconazole 25 % EC. Kiran *et al.*, 2018 findings revealed that Tebuconazole and Hexaconazole were the most effective fungicide in reducing the PDI of *Alternaria* leaf spot of cabbage.

Conclusion

Among fungicides, Tebuconazole 25% EC emerged as the most effective under *in vitro* evaluation, achieving complete growth inhibition. Field trials further validated its efficacy, recording the lowest PDI (6.66%), followed by Trifloxystrobin 25% + Tebuconazole 50% WG (7.66%) and Fluxapyroxad 250 + Pyraclostrobin 250 SC (11.04%), demonstrating their effectiveness in managing the disease. Mancozeb 75% WP and Copper Hydroxide 53.8 % DF, though commonly used, were less effective in reducing disease severity, indicating the need to prioritize systemic and combination fungicides for better disease control.

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