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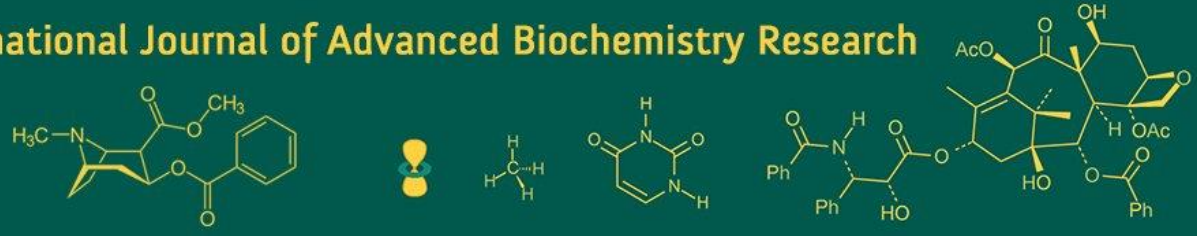

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Management of Alternaria leaf spot of cauliflower caused by *Alternaria brassicicola* (Schwein) Wiltish through new molecule of fungicide

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Abstract
Alternaria leaf spot, caused primarily by *Alternaria brassicicola*, is a major constraint in cauliflower production. In the present study, six new molecule commercially available fungicides were evaluated for their efficacy against *A. brassicicola* through poisoned food technique (*in-vitro*) done with pot and field experiments (*in-vivo*). Among the treatments, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG exhibited complete (100%) inhibition of mycelial growth at 250 ppm and consistently outperformed others in reducing disease severity in both pot and field conditions. In pot trials, the lowest Percent Disease Index (PDI) was recorded with Tebuconazole + Trifloxystrobin (3.25%), followed by Azoxystrobin + Mancozeb + Tebuconazole (4.06%). Similarly, in field trials, the same treatments gave PDI of 3.37% and 4.49%, respectively, after the second spray. Carbendazim 12% + Mancozeb 63% WP was the least effective treatment among all treatments. The findings suggest that strobilurin-triazole fungicide combinations offer superior protection and can serve as an integral component of an integrated disease management strategy for sustainable control of Alternaria leaf spot in cauliflower.

Keywords: *Alternaria brassicicola*, cauliflower, fungicide efficacy, trifloxystrobin, tebuconazole, disease management, strobilurin-triazole, PDI

Introduction
Cauliflower (*Brassica oleracea* L. var. *botrytis*), a major winter vegetable crop of the family Brassicaceae, is believed to have originated in the northeastern Mediterranean region (Gopalkrishnan, 2007) [9]. The name is derived from Latin—*caulis* (cabbage) and *floris* (flower)—reflecting its floral morphology. It evolved from wild cabbage through natural mutation and selection (Valvi *et al*., 2019) [23]. In India, cauliflower is extensively cultivated from September to May due to the development of heat-tolerant varieties. The edible portion is the white curd, a hypertrophied pre-floral apical meristem (Dhaliwal and Vishwakarma, 2017) [6]. Rich in proteins, carbohydrates, vitamins (notably B and C) and minerals, cauliflower is consumed in various forms such as curries, soups and pickles (Pandey and Rizvi, 2009) [17].
India is the world's second-largest producer of cauliflower after China (Sharma *et al*., 2004). During 2021-22, national production reached 89.41 lakh metric tonnes (Anon., 2024) [1].
In Chhattisgarh, the crop cultivated over 24,250 hectares, with a production of 483,565 metric tonnes and an average productivity of 19.94 t/ha. (Anon., 2024) [2].
Among the biotic stresses, Alternaria leaf spot—caused primarily by *Alternaria brassicicola*, *A. brassicae* and occasionally *A. alternata*—is one of the most destructive diseases in cauliflower (Kolte, 1985; Verma and Saharan, 1994; Kohl *et al*., 2010) [13, 24, 12]. The disease affects all growth stages and is known globally by various names such as black spot, brown rot and curd blight. Severe outbreaks are common in subtropical and temperate regions, especially during curd development and seed production stages, resulting in significant yield losses (Kolte *et al*., 1987; Prasad and Vishnuvat, 2006) [14, 18]. In India, it recurs annually from October to February, making it a persistent challenge.
Management of this disease typically involves no availability of effective along with specific fungicide and repeated application of synthetic fungicides, which poses several concerns including environmental pollution, residue accumulation, toxicity to non-target organisms

and the development of fungicide resistance. Hence, the present investigation was undertaken to explore the efficacy of new-generation and combination fungicides for effective and sustainable disease management.

Materials and Methods

A field experiment was conducted during the *Rabi* season of 2024-25 at the Research cum Instructional Farm of Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur, Chhattisgarh. Concurrently, laboratory trials were carried out in the Plant Pathology Laboratory of the same institution. The experimental site is situated in the Southern Bastar Plateau agro-climatic zone of Chhattisgarh.

A survey was conducted to assess the incidence of leaf spot in cauliflower across five blocks of Bastar district, Chhattisgarh. The highest disease incidence was recorded in Bhadisgaon (70.00%) of Tokapal block, while the lowest was observed in Jharumargaon (20.00%) of Bakawand block. Pathogenicity of fifteen *Alternaria* spp. isolates, collected from naturally infected cauliflower plants, was confirmed using the spore suspension technique. Among these, the Alt Jh Ca isolate of *Alternaria brassicicola* was found to be the most virulent. Following the pathogenicity test, the isolates were characterized for their cultural and morphological variability, which revealed features consistent with *Alternaria brassicicola*. The most virulent isolate, Alt Jh Ca was further selected for poisoned food technique in *in-vitro* condition.

In-vitro evaluation of chemical fungicides against *Alternaria brassicicola* isolate Alt Jh Ca

The efficacy of systemic and contact fungicide evaluated at different concentrations *viz.*, 250, 500 and 1000 ppm against *Alternaria brassicicola* isolate Alt Jh Ca in the laboratory conditions by "Poison food technique" (Grover and Moore, 1962) [10].

Double strength potato dextrose agar (PDA) medium was prepared by doubling the quantities of all ingredients except distilled water. The medium was sterilized at 121.6^{0}C in 15 lb psi pressure for 20 minutes. Simultaneously, fungicide stock solutions were prepared in sterilized distilled water to double the desired concentration. Six commercially available fungicides Copper Oxychloride 50% WP, Metiram 55% + Pyraclostrobin 5% WG, Fluxapyroxad 250 G/L +Pyraclostrobin 250 G/L SC, Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG, Carbendazim 12% + Mancozeb 63% WP and Tebuconazole 50% + Trifloxystrobin 25% WG were evaluated under *in-vitro* condition for their efficacy in inhibiting the growth of *A. brassicicola*. The solutions of above fungicides were aseptically mixed with an equal volume of double strength PDA to achieve the required final concentration. The fungicide-amended media were then poured into sterile Petri plates. For each treatment, a 5 mm diameter mycelial disc was cut from the periphery of a three-day-old actively growing culture of the test fungus and placed at the center of each plate. A control was maintained by adding sterile distilled water in place of fungicide solution. All treatments were replicated three times with seven treatments in a Completely Randomized Design (CRD) and incubated at 25 ± 2^{0}C in a BOD incubator. The radial growth (colony diameter) of the test fungus was measured periodically until the control plates were fully covered with fungal growth. The per cent inhibition of mycelial growth at different test concentrations in relation to control was calculated by using the formula:

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

Where:
I = Inhibition of mycelial growth (%)
C = Growth of pathogen in control (mm)
T = Growth of pathogen in treatment (mm)

In-vivo evaluation of fungicides against Alternaria leaf spot disease of Cauliflower

Field Experiment

On the promising results in *in-vitro* condition, field trials were conducted to assess the efficacy of fungicides i.e. T_1 - Copper Oxychloride 50% WP at a concentration of 0.2% (3.0 ml/l of water), T_2 - Metiram 55% + Pyraclostrobin 5% WG at 0.1% (1.0 g/l), T_3 - Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC at 0.04% (0.4 ml/l), T_4 - Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG at 0.4% (4.0 g/l), T_5 - Carbendazim 12% + Mancozeb 63% WP at 0.15% (1.5 g/l) and T_6 - Tebuconazole 50% + Trifloxystrobin 25% WG at 0.05% (0.5 g/l). T_7 was served as the untreated control.

The field trial was laid out in a Randomized Block Design (RBD) comprising seven treatments with three replications (Table 3). The susceptible cauliflower variety 'CFL-1522' was transplanted on 14th December 2024. Each experimental plot was maintained by measuring 4 meter length and 2 meter width in size, with a plant spacing of 50 × 50 cm. Two foliar applications of recommended dose of fungicides were carried out: the first spray was applied upon the initial appearance of disease symptoms and the second was administered in 15 days later. After first spray disease severity was recorded at 3,5,7 and 10day intervals after spraying of fungicides and calculated into PDI (%) along with the percent disease reduction over control. The disease incidence (%) was calculated by the formula:

Disease incidence (%) = (Number of plants/Total number of plants) x 100.

Per cent disease index

$$(PDI) = \frac{\text{Sum of the scores}}{\text{Number of observations} \times \text{Highest number in rating scale}} \times 100$$

Pot Experiment

The Six fungicides were also taken from *in-vitro* assessment and subjected to controlled conditions in polyhouse at Research cum Instructional Farm of Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur, Chhattisgarh. The Pot experiment was laid out in Completely Randomized Design (CRD) with three replication and 7 treatment including control in plastic pots which were 25 cm diameter, 19 cm height and 18.5 cm base diameter in size. All the pots were filled with sterilized mixture of 70% garden and 30% farm yard manure using the susceptible variety "CFL- 1522" of cauliflower. 30 days old seedlings were transplanted in each pot and watered regular intervals. After seven days of transplanting, the plants were inoculated with *Alternaria brassicicola* isolate Alt Jh Ca having CFU 5 x 10^4/ml. Foliar spray of the recommended dose of fungicides was applied when first disease symptoms

appeared on the plants. The observations on disease severity were recorded and calculated into PDI (%).
The data on the severity of the disease were recorded by using 0-5 scale given in Table 1.

Table 1: Scale for assessing disease severity of Alternaria leaf spot disease of cauliflower

Rating	Infected leaf area (%)	Disease reaction
0	No visible infection	Immune (I)
1	1-5.0	Resistance (R)
2	5.1-15.0	Moderately resistant (MR)
3	15.1-30.0	Moderately susceptible (MS)
4	30.1-50.0	Susceptible (S)
5	>50.0	Highly susceptible (HS)

Where, I= Immune, R = Resistant. MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible and HS = Highly susceptible

Results and Discussion

Poison food technique

The results of this study are presented in Table 2. The efficacy of six fungicidal formulations against *Alternaria brassicicola* isolate Alt Jh Ca was assessed under *in-vitro* conditions, using the poisoned food technique, all fungicides were tested at 250, 500 and 1000 ppm concentrations. At 250 ppm, the complete inhibition of (100%) mycelial growth was observed in Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG and Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC. On the other hand, Copper Oxychloride 50% WP, Metiram 55% + Pyraclostrobin 5% WG and Carbendazim 12% + Mancozeb 63% WP showed lower inhibition (78.88%, 91.11% and 91.66%, respectively). The complete inhibition percent of mycelial growth was recorded in fungicides Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG and Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC except Carbendazim + Mancozeb (81.11%) and Copper Oxychloride (85.00%) at 500 ppm concentration, while at 1000 ppm concentration, the 100% inhibition of mycelial growth was recorded in almost treatments except Carbendazim + Mancozeb (91.66%) which was showed lower efficacy as compared to other fungicides.
The results are reinforced by the work of Varma *et al*. (2024) [25] in *A. burnsii*, who noted that *in-vitro* application of Hexaconazole, Tebuconazole, Tebuconazole50% +Trifloxy strobin20% respectively were quite effective in inhibiting growth in *Alternaria* spp. and exhibited 100%, 100%, 76.94% mycelial inhibition, respectively while studying their *in-vitro* effect on growth of *Alternaria* spp. Yadav *et al*. (2020) [27] confirmed the efficacy of the combination fungicide Tebuconazole + Trifloxystrobin under both *in-vitro* and field conditions. When applied as a foliar spray, it significantly reduced disease intensity under natural conditions, demonstrating superior effectiveness at both 60 and 90 days after transplanting. Rachel and Christine (2016) [19] reported that the effective concentration of azoxystrobin required to inhibit 50% of spore germination in *Alternaria brassicicola* infecting Brassica crops ranged from 0.22 to 14 µg/mL. Kaki *et al*., (2020) reported that the radial growth of Copper oxychloride @ 1500 ppm observed was 12.50 mm with per cent inhibition of 86.1 per cent. Carbendazim+ Mancozeb recorded the radial growth of 3.43 mm and 96.1 per cent inhibition at 1500 ppm whereas, radial growth @ 2000 ppm was 2.76 with per cent inhibition of 96.9 per cent, which was significantly superior than non-systemic fungicide.
These findings confirm the superior *in-vitro* activity of strobilurin-triazole-based formulations, likely due to their dual modes of action—disruption of mitochondrial respiration and inhibition of ergosterol biosynthesis (Gisi *et al*., 2002; Bartlett *et al*., 2002) [8, 3].

In-vivo (Field) evaluation of fungicides against Alternaria leaf spot disease

The field evaluation of different fungicides to control the *Alternaria* leaf spot disease of cauliflower revealed significant variations in efficacy among the treatments. The Tebuconazole 50% + Trifloxystrobin 25% WG at a dosage of 0.5 g/L was found to be the most effective, recording the lowest percent disease index (PDI) of 3.37% and the highest percent disease reduction (93.35%), which was found at par with Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG (4 g/L), where 4.29% percent disease index (PDI) and 91.54% (PDR) were recorded, whereas Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC at 0.4 ml/L and Metiram 55% + Pyraclostrobin 5% WG at 1 g/L also performed well, showing per cent disease index of 10.06% (18.43) and 11.06% (19.40) and per cent disease reduction (PDRs) of 80.17% and 78.20%, respectively. On the contrary Copper Oxychloride 50% WP (3 g/L) and Carbendazim 12% + Mancozeb 63% WP (1.5 g/L) were moderately effective, recorded PDI values of 13.04% (21.02) and 15.25% (22.96) and corresponding per cent disease reduction (PDRs) of 74.30% and 69.95% respectively. The untreated control (water spray) exhibited the highest disease incidence with a PDI of 50.75%. Observations were taken as per fixed schedule after fungicides spray the results of this study are presented in Table 3.
Similarly, Tebuconazole-based fungicides have consistently demonstrated high efficacy against *Alternaria* spp. under field conditions. Singh *et al*. (2020) [21] found that the combination of Tebuconazole + Trifloxystrobin significantly reduced disease severity of *Alternaria leaf spot* in cruciferous crops, with more than 90% disease suppression. Similarly, Sharma and Meena (2019) [20] reported that Azoxystrobin-based formulations, particularly those combined with Mancozeb and Tebuconazole, showed strong protectant and curative actions, significantly lowering the PDI in cauliflower fields. The efficacy of Fluxapyroxad + Pyraclostrobin and Metiram + Pyraclostrobin mixtures has also been confirmed by Yadav *et al.* (2021) [28], who observed over 70% disease reduction in trials conducted against *Alternaria brassicicola* in mustard and cauliflower. In contrast, Copper Oxychloride and Carbendazim + Mancozeb, although commonly used, were reported as moderately effective, aligning with the present findings (Kumar *et al*., 2018) [15]. The untreated control consistently exhibited the highest disease severity, reaffirming the critical need for effective fungicidal interventions for managing *Alternaria* leaf spot.
These field findings reinforce the systemic and protective properties of strobilurin-triazole combinations, including their translaminar movement and residual activity (Gisi and

Sierotzki, 2008) [8]. Similar observations were reported by Singh *et al*. (2020) [22] in cruciferous crops.

The comparatively poor performance of Carbendazim + Mancozeb may be due to reduced sensitivity or resistance development in *A. brassicicola* populations, as earlier suggested by Brent and Hollomon (2007) [4]. This highlights the necessity of fungicide rotation and adoption of integrated disease management (IDM) strategies. Use of combination fungicides with multiple sites of action, such as Tebuconazole + Trifloxystrobin and Azoxystrobin + Mancozeb + Tebuconazole, not only enhances efficacy but also helps delay resistance buildup (FRAC, 2024) [7].

Table 2: *In-vitro* evaluation of different fungicides against growth of *A. brassicicola* (Alt Jh Ca)

S.N.	Treatment	Mycelial growth (mm)*			Inhibition (%)		
		250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
1	T_1 - Copper Oxychloride 50% WP	19.00	13.50	0.00	78.88	85.00	100.00
2	T_2 - Metiram 55% + Pyraclostrobin 5% WG	8.00	0.00	0.00	91.11	100.00	100.00
3	T_3 - Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC	0.00	0.00	0.00	100.00	100.00	100.00
4	T_4 - Azoxystrobin 4.7% + Mencozeb 59% + Tebuconazole 5.6% WG	0.00	0.00	0.00	100.00	100.00	100.00
5	T_5- Carbendazim 12% + Mencozeb 63% WP	21.00	17.00	7.50	76.66	81.11	91.66
6	T_6 - Tebuconazole 50% + Trifloxystrobin 25% WG	0.00	0.00	0.00	100.00	100.00	100.00
7	T_7 - Control (Water spray)	90.00	90.00	90.00	-	-	-
	C.D. (p>=0.01)	2.27	3.55	2.92			
	SE(m)±	0.64	0.88	0.72			

*Mean of three replications

Table 3: *In-vivo* (Field) evaluation of different fungicides against the Alternaria leaf spot disease of cauliflower

S.N.	Treatments	Dosage (ml or g /litre)	Per cent disease index (PDI)	PDR (%) **
1	T_1 - Copper Oxychloride 50% WP	3g/L	13.04(21.02)	74.30
2	T_2 - Metiram 55% + Pyraclostrobin 5% WG	1g/L	11.06(19.40)	78.20
3	T_3 - Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC	0.4ml/L	10.06(18.43)	80.17
4	T_4 - Azoxystrobin 4.7% + Mencozeb 59% + Tebuconazole 5.6% WG	4g/L	4.29(11.94)	91.54
5	T_5 - Carbendazim 12% + Mencozeb 63% WP	1.5g/L	15.25(22.96)	69.95
6	T_6 - Tebuconazole 50% + Trifloxystrobin 25% WG	0.5g/L	3.37(10.53)	93.35
7	T_7 - Control (water spray)	-	50.75(45.41)	
	C.D. (p>=0.05)		2.34	
	SE(m)±		0.78	
	C.V.		7.30	

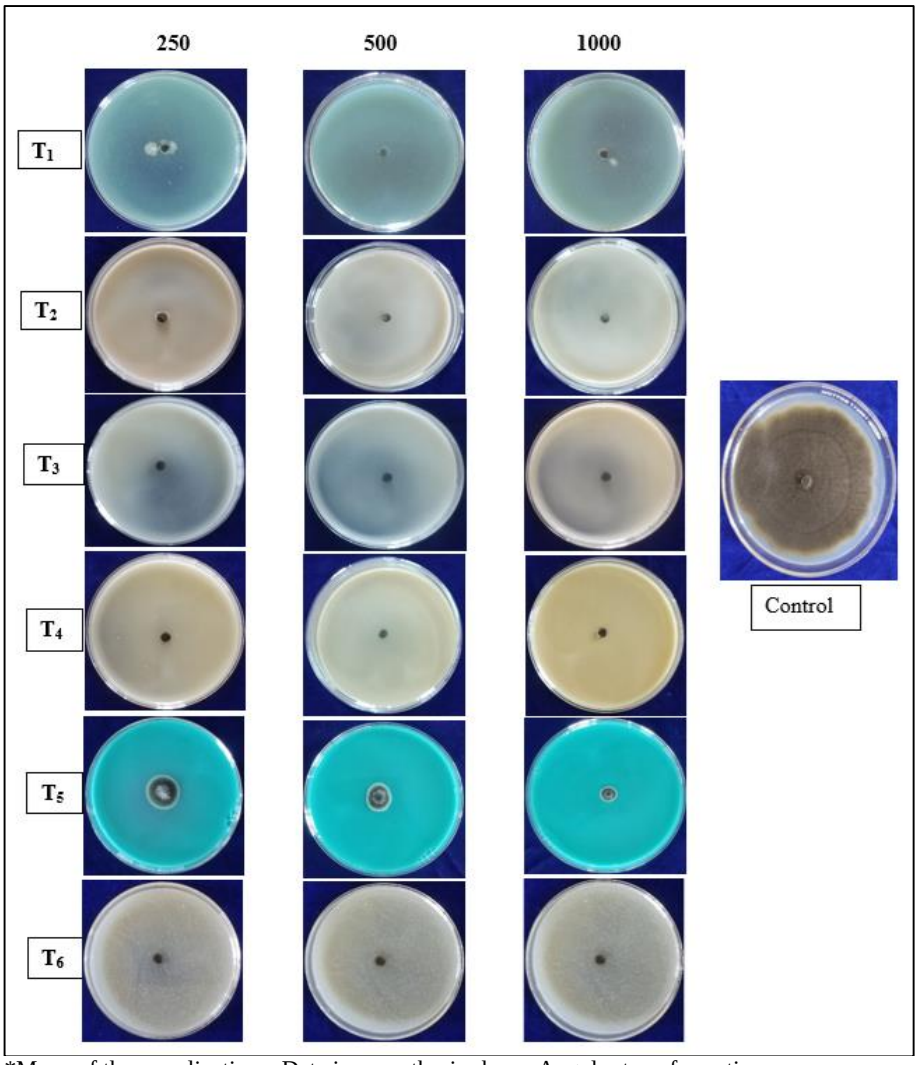
*Mean of three replications; Data in parenthesis shows Angular transformation

*PDR is Percent Disease Reduction

In-vivo (Pot) evaluation of fungicides against Alternaria leaf spot disease

The results of this study are presented in Table 4. Under natural pot conditions, significant differences in disease suppression were observed among treatments. Tebuconazole + Trifloxystrobin was recorded in the lowest Percent Disease Index (PDI) of (3.25%), followed by Azoxystrobin + Mancozeb + Tebuconazole (PDI: 4.06%), which was found at par with each. In contrast, the highest disease severity (15.59%) was observed with Carbendazim + Mancozeb. The Percent Disease Reduction (PDR) over control followed a similar trend, with Tebuconazole + Trifloxystrobin (92.52%) and Azoxystrobin + Mancozeb + Tebuconazole (90.66%) outperforming other treatments.

Similarly, under controlled environments, Tebuconazole + Trifloxystrobin has consistently shown superior efficacy against *Alternaria* spp., significantly reducing disease severity. Meena *et al*. (2020) [16] reported that under pot culture conditions, this combination provided over 90% disease suppression in cauliflower plants. Similarly, Verma and Singh (2019) [26] observed that the fungicidal mixture of Azoxystrobin + Mancozeb + Tebuconazole effectively minimized Alternaria leaf spot under pot experiments, owing to its broad-spectrum activity and systemic movement within plant tissues. These outcomes are comparable to the present study, where both combinations recorded the lowest Percent Disease Index (PDI) and highest Percent Disease Reduction (PDR) over control. In contrast, Carbendazim + Mancozeb exhibited comparatively higher disease incidence, which aligns with the findings of Choudhary *et al.* (2018) [5], who reported limited effectiveness of this combination under pot conditions, possibly due to reduced systemic absorption or emerging fungicide resistance. These results reaffirm the importance of selecting fungicides with both protective and curative properties, especially in controlled pot experiments simulating natural infection pressure.



*Mean of three replications; Data in parenthesis shows Angular transformation
*PDR is Percent Disease Reduction

Plate 1: *In-vitro* evaluation of different fungicides against growth of *A. brassicicola*.

Table 4: *In-vivo* (Pot) evaluation of different fungicides against Alternaria leaf spot of cauliflower

S.N.	Treatment	Dosage	Per cent disease index (PDI)*	PDR (%) **
1	T_1 - Copper Oxychloride 50% WP	3g/L	14.13(22.06)	67.51
2	T_2 - Metiram 55% + Pyraclostrobin 5% WG	1g/L	11.79(20.05)	72.89
3	T_3 - Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L SC	0.4ml/L	11.13(19.47)	74.41
4	T_4 - Azoxystrobin 4.7% + Mencozeb 59% + Tebuconazole 5.6% WG	4g/L	4.06(11.57)	90.66
5	T_5 - Carbendazim 12% + Mencozeb 63% WP	1.5g/L	15.59(23.24)	64.16
6	T_6 - Tebuconazole 50% + Trifloxystrobin 25% WG	0.5g/L	3.25(10.36)	92.52
7	T_7- Control (Water spray)		43.50(41.24)	-
	C.D. (p>=0.01)		1.48	
	SE(m)±		0.49	
	C.V.		4.69	

Conclusion

The present study demonstrated that among the six evaluated fungicides, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 4.7% + Mancozeb 59% + Tebuconazole 5.6% WG were the most effective in managing the *Alternaria brassicicola* and leaf spot disease both under *in-vitro* and *in-vivo* conditions respectively. These fungicides consistently achieved maximum mycelial growth inhibition in poisoned food assays and significantly reduced disease severity in pot and field trials, indicating their superior systemic and protective properties. In contrast, Carbendazim 12% + Mancozeb 63% WP was the least effective, suggesting limited efficacy or emerging resistance in pathogen populations.

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