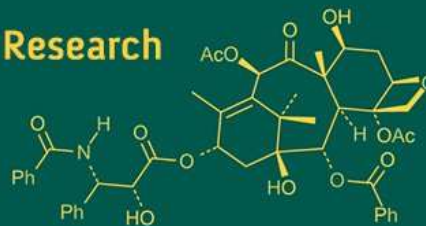
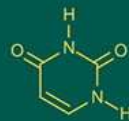
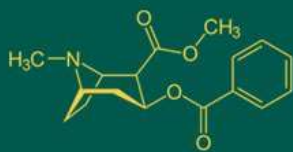


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(6): 1555-1562
www.biochemjournal.com
Received: 30-04-2026
Accepted: 31-05-2026

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Efficacy of novel fungicide combinations against downy mildew in cucumber and their influence on biochemical defense mechanisms

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6Sr.8879>

Abstract

Downy mildew, caused by *Pseudoperonospora cubensis*, severely impacts cucumber (*Cucumis sativus* L.) in humid regions like North Gujarat, prompting evaluation of chemical fungicides as alternatives to resistant metalaxyl and copper-based options. A field trial was conducted in summer 2023 at Vegetable Research Station, Sardarkrushinagar Dantiwada Agricultural University, SDAU, used Randomized Block Design with eight treatments: Ametoctradin 27 SC + Dimethomorph 20.27 SC (0.2%), Metalaxyl 8 + Mancozeb 64 WP (0.2%), Cymoxanil 8 + Mancozeb 64 WP (0.2%), Chlorothalonil 33.1 + Metalaxyl M 3.3 SC (0.7%), Dimethomorph 50 WP (0.1%), Metiram 55 + Pyraclostrobin 50 WG (0.2%), Azoxystrobin 23 SC (0.25%) and untreated control. Sprays at 500 L/ha were applied post-disease onset, with assessments of percent disease intensity, area under disease progress curve, fruit yield (q/ha) and biochemicals (peroxidase, polyphenol oxidase, chlorophyll). Metalaxyl 8 + Mancozeb 64 WP excelled with lowest PDI (22.76%), 61.75% disease reduction, AUDPC (380.80 units) and highest yield (153.43 q/ha; 191.14% increase over control). Comparable results came from Ametoctradin + Dimethomorph (PDI 24.32%, yield 143.93 q/ha) and Cymoxanil + Mancozeb (PDI ~25%, yield 146.51 q/ha). All fungicides significantly outperformed control (PDI up to 59%, AUDPC 1007.03, yield 80.27 q/ha). Biochemical assays showed elevated peroxidase (0.18 U/g FW, Metalaxyl combo), PPO (0.18 U/g FW) and chlorophyll (1.83 mg/g FW), peaking 1-5 days post-spray before slight decline at 10 days, indicating induced defense activation. These fungicides offer sustainable management, minimizing resistance and phytotoxicity risks. Metalaxyl-Mancozeb is recommended for cucumber downy mildew control, enhancing yield and host defenses in tropical climates.

Keywords: Cucumber (*Cucumis sativus* L.), Downy mildew, *Pseudoperonospora cubensis*, Fungicide efficacy

Introduction

Cucumber (*Cucumis sativus* L.) is important vegetable crop which belongs to gourd family Cucurbitaceae and consisting of 118 genera and 825 species (Lebeda and Cohen, 2011) [6]. It is the second most popular cucurbit after watermelon and the most extensively planted crop after tomatoes, cabbage and onions (Tatlioglu, 1993) [15]. It grows well in the world's tropical and subtropical regions as well as plains and hills (Shetty *et al.*, 2002) [12]. India is the primary origin of cucumbers. Cucumber farming dates back 2000 years in China and 3000 years in India, according to archaeological data. According to De Candolle (1882) [5], China is regarded as a secondary centre of cucumber genetic diversification. Vitamins "B" and "C" and minerals including calcium, phosphorus, iron and potassium are abundant in cucumber. Silica, a trace mineral that fortifies connective tissues, is abundant in it. Because it includes 95% water, it helps rehydrate the entire body and meets daily vitamin requirements. Cucumber contains 96.3 g of fluids, 0.4 g of proteins, 0.1 g of fat, 0.3 g of minerals, 0.4 g of dietary fibre, 2.4 g of carbohydrates, 10 mg of calcium, 25 mg of phosphorus, 1.5 mg of iron, 0.03 mg of thiamine, 0.2 mg of niacin, 7 mg of vitamin "C" and 13 K cal. (Yadav and Rai, 2005) [9]. Cucumbers are now grown in greenhouses, small gardens and commercial farms of all sizes all over the world (Hossain *et al.*, 2010). Over 75% of the world's cucumber production comes from China. India produces 1651.92 thousand Mt of cucumbers on 116.62 thousand hectares of land (Anon., 2020-21).

West Bengal (322.29 Mt), Madhya Pradesh (252.26 Mt) and Karnataka (122.98 Mt) are the three states that produce the most cucumbers. These three states account for approximately 41.21 percent of the nation's land area and 42.22 percent of its production (Anon., 2020-21).

A number of diseases brought on by bacteria, viruses and fungi afflict cucumbers. One of the most economically significant and pervasive plant infections in many parts of the world is *Pseudoperonospora cubensis* (Berkeley and Curtis), which causes downy mildew of cucumbers (Palti and Cohen, 1980) [8]. The disease is found everywhere cucurbits are grown, including in semi-arid regions of the Middle East as well as temperate and tropical regions. The infection has a significant impact on both quality and yield in these producing zones and it is particularly harmful in places with warm, humid weather that is conducive to disease growth (Thomas, 1996) [16]. It is estimated that downy and powdery mildew infections cause a 50-70% yield loss (Sitterly, 1972) [13]. Crop loss was reported to be between 50 and 70 percent despite the application of fungicides, crop rotation and fall ploughing, among other cultural measures (Awad, 2000) [4]. During the 1998 crop season, the incidence and magnitude of downy mildew varied from 50 to 100% and 20 to 78%, respectively (Sharma *et al.*, 2003) [11]. According to Vyavahare (2020) [17], the incidence of downy mildew in various Maharashtra districts was between 11.87 and 48.47 percent in 2018 and 19.56 and 60.13 percent in 2019.

Downy mildew is most problematic in moist and humid climate of North Gujarat; generally, cucurbits are grown in large scale throughout the year. Therefore, due to intensified cropping of cucurbits, downy mildew causes havoc losses.

Farmers of North Gujarat prefer either age old metalaxyl and copper-based fungicides or some systemic fungicides. This experiment of the disease is of practical significance, since the pathogen disseminates through air, disease spreads very fast. Hence, identification of the natural factors to assess the infection to the crop plant and its management through the novel fungicide available in Indian market.

Materials and Methods

In order to manage cucumber downy mildew in the field, the current study was conducted in the summer of 2023 at the Vegetable Research Station, SDAU, and Sardarkrushinagar. Eight treatments, including the control, have been incorporated in the Randomised Block Design experimental design employed for the study. For every experiment treatment, a 5 x 6 m² plot was maintained. There was total eight treatments including the control. The application of fungicides was done at 15 days interval. Where, first spray was done on the first appearance of disease. The total seven fungicides tested together for their efficacy those are: Ametoctradin 27% SC + Dimethomorph 20.27% SC @ 0.2%, Metalaxy 18% + Mancozeb 64% WP @ 0.2%, Cymoxil 8% + Mancozeb 64% WP @ 0.2%, Chlorothalonil 33.1% + Metalaxyl M 3.3% SC @ 0.7%, Dimethomorph 50 WP @ 0.1%, Metiram 55% + Pyraclostrobin 50% WG @ 0.2% and Azoxystrobin 23 SC @ 0.25%. Water was the only substance utilised in the control plot. Details of the treatment are provided in Table 1. Fruits were picked and block-by-block observations were made to assess each treatment's yield and B:C ratio independently. Both treated and untreated plots' yields were noted and calculated in tonnes/ha.

Table 1: List of fungicides used for management of cucumber downy mildew

Treatment details	Fungicides	
	T ₁	Ametoctradin 27% SC + Dimethomorph 20.27% SC
	T ₂	Metalaxy 18% + Mancozeb 64% WP
	T ₃	Cymoxil 8% + Mancozeb 64% WP
	T ₄	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC
	T ₅	Dimethomorph 50 WP
	T ₆	Metiram 55% + Pyraclostrobin 50% WG
	T ₇	Azoxystrobin 23 SC
	T ₈	Control (no spray)

Five randomly chosen plants per plot were used to measure the prevalence of downy mildew in order to assess the effectiveness of treatments. The observations were obtained on the 7th, 14th, 21st, 28th and 35th days following the initial spray, as well as before the 24-hour mark.

Percent disease intensity

The following formula provided by Wheeler (1969) [18] was used to calculate the % disease intensity of downy mildew.

$$\text{PDI} = \frac{\text{Sum of numeral rating}}{\text{Total no. of samples observed} \times \text{Maximum of rating scale}} \times 100$$

Percentage disease control

The percentage disease control (PDC) was calculated according to formula given by (Mathur *et al.*, 1971) [17].

$$\text{PDC} = \frac{\text{PDI in Control} - \text{PDI in treatment}}{\text{PDI in control}}$$

Area under disease progress curve (AUDPC)

Using the method provided by Shaner and Finney (1977) [10], the plant disease per cent disease index was determined at seven-day intervals to determine AUDPC.

$$\text{AUDPC} = \sum_{i=1}^n \{(X_i + X_{i+1}) \times t_i\} / 2$$

Where,

X_i and X_{i + 1} are severities on date i and date i + 1, respectively

t_i is the number of days in between date i and date

i + 1 n is the number of observations recorded.

Biochemical analysis

To determine how the fungicidal treatment affected the activity of these biochemicals, biochemical analyses of peroxidase, polyphenol oxidase and chlorophyll content were conducted before and after spraying at intervals of one, five, and ten days. Peroxidase, polyphenol oxidase, and chlorophyll activity were measured using methods described by Yadav *et al.* (2018) ^[19], Taneja and Sachar (1974) ^[14] and Arnon (1949) ^[3]. The relevance of the final evaluations was then determined using statistical analysis. The computer program "OPSTAT," created by O.P. Sheoran, CCS HAU, Hisar, was also used to investigate the data collected throughout the research.

Result and discussion

Per cent disease intensity (pdi)

All of the fungicides tested in field conditions considerably decreased the intensity of downy mildew infection as compared to control, according to a review of the data shown in table 2 and fig 4.3 (a). Metalaxyl 8% + Mancozeb 64% WP had the lowest PDI i.e., 22.76, whereas ametoctradin 27% SC + dimethomorph SC, metiram 55% + pyraclostrobin 50% WG, chlorothalonil 33.1% + metalaxyl M 3.3% SC, and dimethomorph had disease intensities of 24.32, 25.07, 26.25, 26.91 and 27.32 percent and disease reductions of 61.75, 60.57, 58.71, 57.67 and 56.99 percent, respectively. These therapies were found to be comparable to one another. Azoxystrobin 23 SC had the lowest disease reduction (54.91%) above control and the highest disease intensity (28.67%).

Area under disease progress curve

All of the fungicides tested in field conditions considerably decreased downy mildew disease when compared to control in terms of area under the disease progress curve, according to a review of the data shown in table 4.4 and figure 4.3 (c). The treatment with the lowest AUDPC was metalaxyl 8% + mancozeb 64% WP 0.20% (380.80 units), which was comparable to ametoctradin 27% SC + dimethomorph 20.27% SC (446.98 units) and cymoxil 8% + mancozeb 64% WP 0.20% (432.25 units). Chlorothalonil 33.1% + metalaxyl M 3.3% SC 0.20% (479.57 units), dimethomorph 50 WP 0.1% (481.77 units), azoxystrobin 23 SC 0.03% (495.42 units), and metiram 55% + pyraclostrobin 50% WG (462.59 units) were the next best treatments in merit. Every treatment was comparable to the others. The control plot has the highest AUDPC value (1007.03 units).

Fruit yield (q/ha)

All of the treatments were found to be significantly better in terms of fruit yield, according to the data shown in table 2. Plots treated with metalaxyl 8% + mancozeb 64% WP 0.2% achieved the highest fruit yield of 153.43 q/ha with a maximum yield increase of 191.14% over the control plot,

and cymoxil 8% + mancozeb 64% WP 0.2% produced 146.51 q/ha with a yield increase of 182.52 over control, which was comparable to ametoctradin 27% SC + dimethomorph 20.27% SC 0.2%, which recorded 143.93 q/ha with a yield enhancement of 179.30 over control. Metiram 55% + pyraclostrobin 50% WG, chlorothalonil 33.1% + metalaxyl M 3.3% SC 0.20%, dimethomorph 50 WP 0.1% and azoxystrobin 23 SC 0.03% were the next best treatments in merit. Their equivalent yield increases were 165.69, 162.12, 155.98 and 151.43 percent over control and 133.00 q/ha, 130.14 q/ha, 125.22 q/ha and 121.54 q/ha, respectively. 80.27q/ha was the lowest yield observed in the control plot.

Biochemical analysis

Peroxidase activity (u g-1 fresh weight of tissue)

Following treatment spraying, peroxidase activity was measured in each treatment and ranged from 0.13 U gm⁻¹ FW to 0.18 U gm⁻¹ FW. The leaves sprayed with metalaxyl 8% + mancozeb 64% WP (0.18 Ugm⁻¹ FW) had the highest peroxidase activity, whereas the plot treated with fungicide dimethomorph 50% WP (0.11 U gm⁻¹ FW) had the lowest. In contrast to the control, the other treatments had demonstrated notable variations. In all treatments, the range of activity increased one day and five days after spraying, while the peroxidase activity showed a little decrease ten days after spraying, as indicated in table 3 and figure 2.

Polyphenol oxidase activity

Following treatment spraying, the polyphenol oxidase activity in each treatment was measured and found to vary between 0.10 and 0.18 U gm⁻¹ FW. The leaves treated with metalaxyl 8% + mancozeb 64% WP had the highest peroxidase activity (0.18 U gm⁻¹ FW), whereas the leaves treated with azoxystrobin 23% SC had the lowest (0.10 U gm⁻¹ FW). In contrast to the control, the other treatments had demonstrated notable variations. In all treatments, the range of activity increased one day prior to and five days following spraying, while the polyphenol oxidase activity slightly decreased ten days following spraying, as indicated in table 4 figure 3.

Chlorophyll content

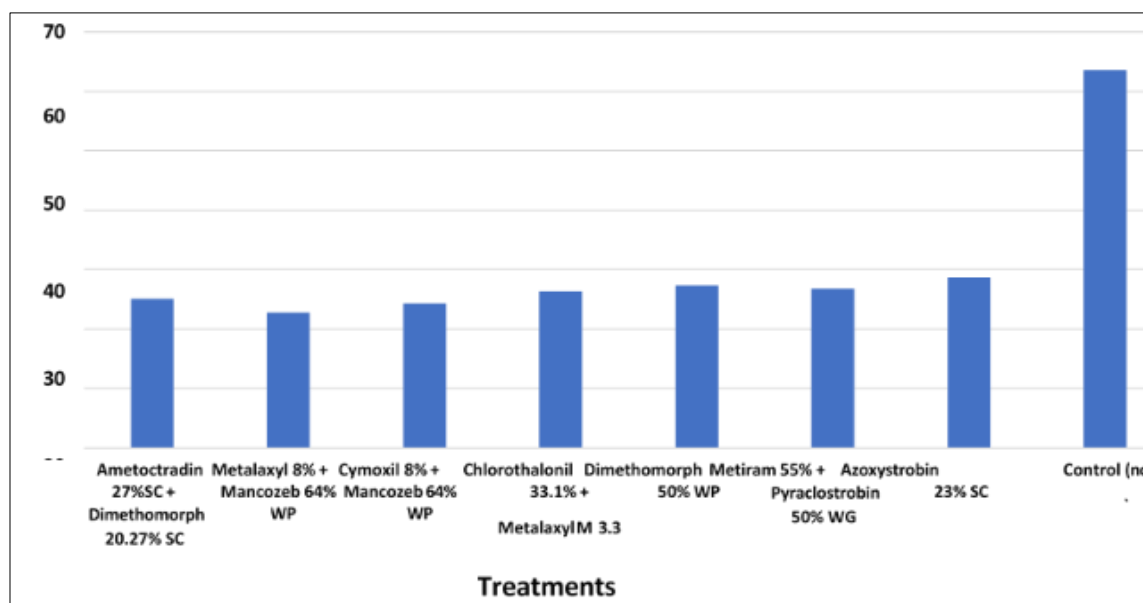
When chemical treatments were used in the field following disease incidence, the amount of chlorophyll increased slightly compared to what was seen before to the chemical spray. The plot sprayed with metalaxyl 8% + mancozeb 64% WP (1.83 mg/g fresh leaf) had the highest chlorophyll content, whereas plants treated with azoxystrobin 23 SC (1.69 mg/g fresh leaf) had the lowest. Significant variations in the chlorophyll content were also observed in the other treatments when compared to the control, as indicated in table 5 and figure 4.

Table 2: Per cent disease intensity at different interval after spray of ecofriendly treatments

Tr. No.	Treatments	7 DAS	14 DAS	21 DAS	28 DAS	35 DAS
1	Ametoctradin 27% SC + Dimethomorph 20.27% SC	14.45 ^{bcd} (6.23)	18.65 ^b (10.23)	21.70 ^{bcd} (13.67)	26.87 ^{bcd} (20.42)	30.05 ^{bc} (25.07)
2	Metalaxyl 8% + Mancozeb 64% WP	13.78 ^d (5.67)	16.56 ^c (8.12)	19.98 ^d (11.67)	24.78 ^d (17.56)	28.50 ^c (22.76)
3	Cymoxil 8% + Mancozeb 64% WP	14.08 ^{cd} (5.92)	19.17 ^b (10.78)	22.42 ^{bc} (14.54)	25.36 ^{cd} (18.34)	29.56 ^{bc} (24.34)
4	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC	15.00 ^{bc} (6.70)	20.32 ^b (12.06)	22.70 ^{bc} (14.89)	27.78 ^{bc} (21.72)	31.25 ^{bc} (26.91)
5	Dimethomorph 50 WP	15.27 ^b (6.94)	19.88 ^b (11.56)	22.16 ^{bc} (14.23)	28.74 ^b (23.12)	31.51 ^{bc} (27.32)
6	Metiram 55% + Pyraclostrobin 50% WG	13.79 ^d (5.68)	19.58 ^b (11.23)	21.10 ^{cd} (12.96)	27.18 ^{bcd} (20.86)	30.82 ^{bc} (26.25)
7	Azoxystrobin 23 SC	15.48 ^b (7.12)	20.46 ^b (12.22)	23.39 ^b (15.76)	28.21 ^b (22.34)	32.37 ^b (28.67)
8	Control (no spray)	18.64 ^a (10.21)	32.15 ^a (28.32)	37.06 ^a (36.32)	39.95 ^a (41.23)	52.88 ^a (63.57)
	S. Em. (±)	0.30	0.61	0.75	1.05	1.43
	C. D. at 5%	0.92	1.85	2.28	3.19	4.33
	C. V. %	7.7	8.1	8.0	8.1	8.1

Figures in parenthesis are original values and outside are transformed values DAS-Days after spray Treatment mean

with the common letter/letters are non-significant by DNMR at 5% level of significance

**Fig 1:** (a) Effect of fungicides on per cent disease intensity**Table 3:** Evaluation of fungicides against cucumber downy mildew under field condition

Sr. No.	Treatment	Conc. (%)	Percent Disease Intensity 35 DAS	Percent Disease Reduction over Control	AUDPC	Yield (Q/ha)	Increase yield over control (%)
1	Ametoctradin 27% SC + Dimethomorph 20.27% SC	0.2%	30.05 ^{bc} (25.07)	60.57	446.98 ^{bc}	143.93 ^{ab}	179.30
2	Metalaxyl 8% + Mancozeb 64% WP	0.2%	28.50 ^c (22.76)	64.20	380.80 ^c	153.43 ^a	191.14
3	Cymoxil 8% + Mancozeb 64% WP	0.2%	29.56 ^{bc} (24.32)	61.75	432.25 ^{bc}	146.51 ^a	182.52
4	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC	0.073%	31.25 ^{bc} (26.91)	57.67	479.57 ^b	130.14 ^{bc}	162.12
5	Dimethomorph 50 WP	0.1%	31.51 ^{bc} (27.32)	56.99	481.77 ^b	125.22 ^{bc}	155.98
6	Metiram 55% + Pyraclostrobin 50% WG	0.2%	30.82 ^{bc} (26.25)	58.71	462.59 ^b	133.00 ^{bc}	165.69
7	Azoxystrobin 23 SC	0.025%	32.37 ^b (28.67)	54.91	495.42 ^b	121.54 ^{bc}	151.43
8	Control (no spray)	-	52.88 ^a (63.57)	-	1007.05 ^a	80.27 ^c	-
	S. Em. (±)		1.11	-	24.19	5.88	-
	C. D. at 5%		3.38	-	73.37	17.83	-
	C. V. %		8.3	-	8.0	7.9	-

Figures in parenthesis are original values and outside are transformed values Treatment mean with the common

letter/letters are non-significant by DNMR at 5% level of significance

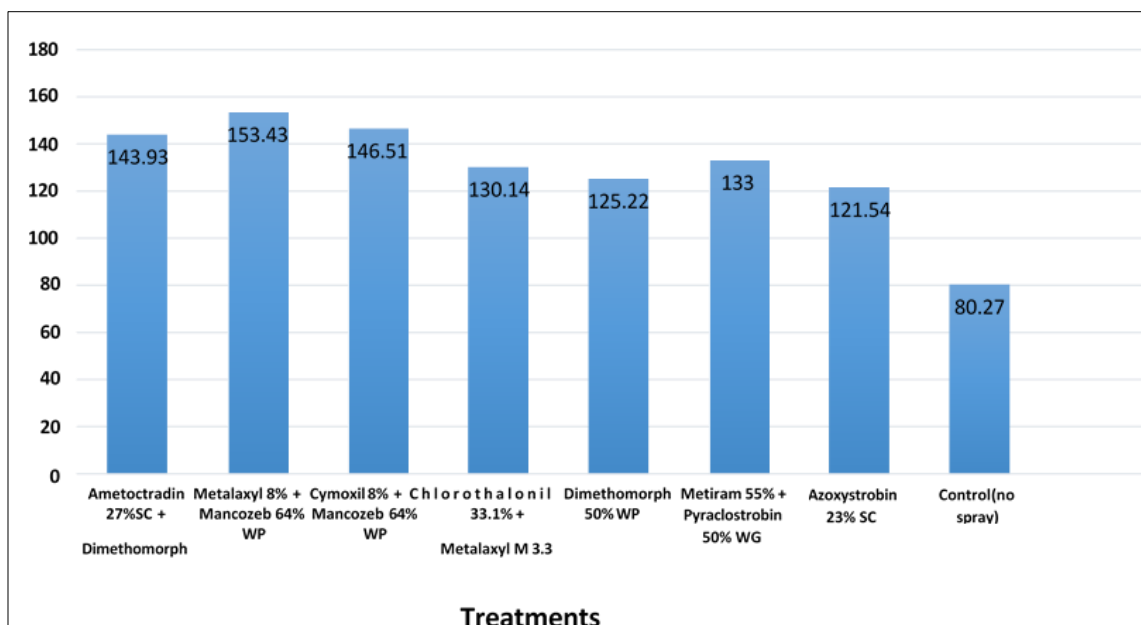


Fig 2: (b): Effect of fungicides on fruit yield of cucumber

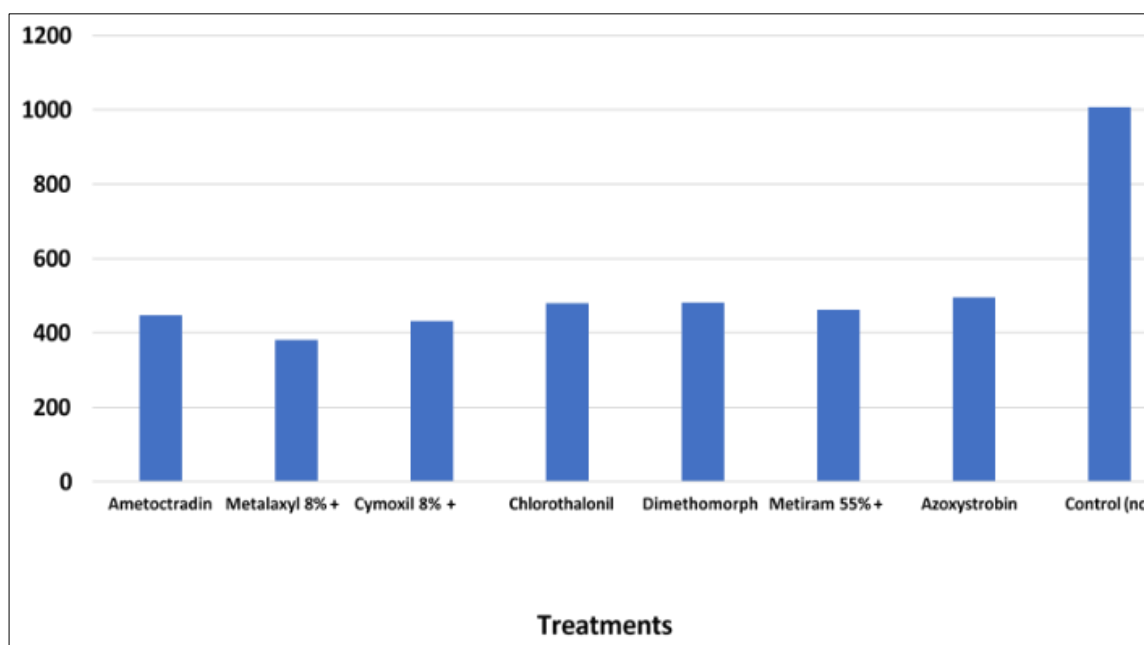


Fig 3: (c): Effect of fungicides on AUDPC of downy mildew of cucumber

Table 3: Effects of fungicidal treatments on the peroxidase activity

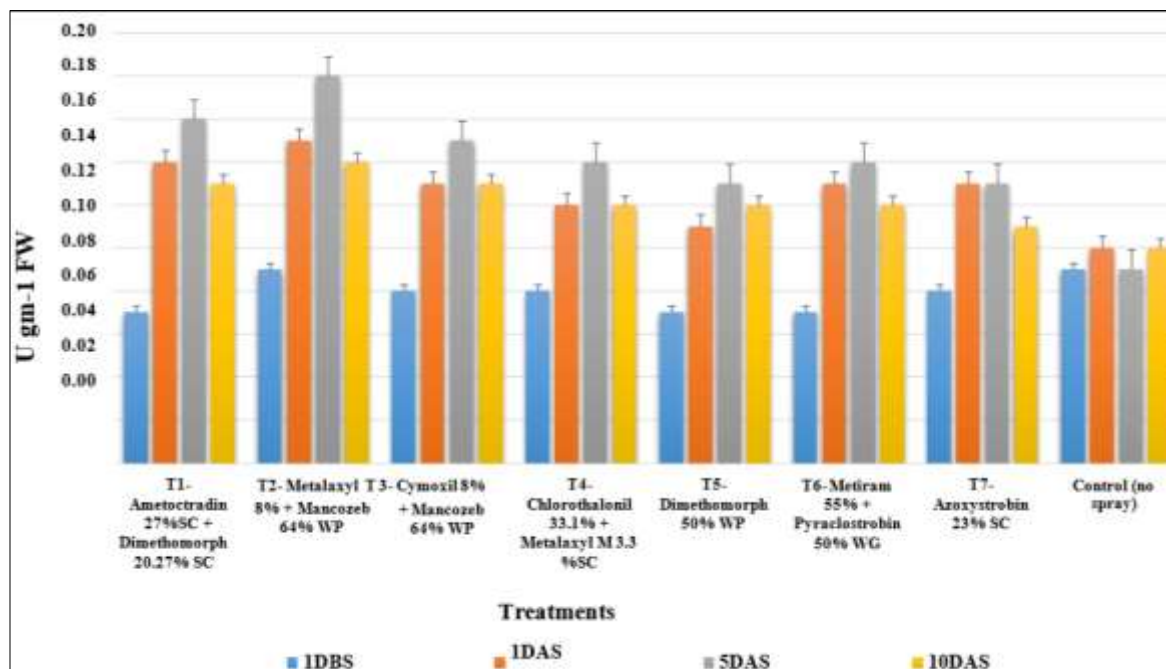
Peroxidase activity (Ugm-1 FW)					
Sr. No.	Treatments	1DBS	1DAS	5DAS	10DAS
1	Ametoctradin 27% SC + Dimethomorph 20.27% SC	0.07	0.15	0.16	0.13
2	Metalaxyl 8% + Mancozeb 64% WP	0.09	0.15	0.18	0.14
3	Cymoxil 8% + Mancozeb 64% WP	0.08	0.13	0.15	0.13
4	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC	0.08	0.12	0.14	0.12
5	Dimethomorph 50 WP	0.07	0.11	0.13	0.12
6	Metiram 55% + Pyraclostrobin 50% WG	0.07	0.13	0.14	0.12
7	Azoxystrobin 23 SC	0.08	0.13	0.13	0.11
8	Control (no spray)	0.09	0.10	0.09	0.10
S. Em (±)		0.002	0.003	0.004	0.003
C. D. at 5%		0.01	0.01	0.01	0.01
C. V.%		4.56	4.75	4.65	4.83

Table 4: Effects of the fungicidal treatments on the polyphenol activity

Polyphenol oxidase activity (U gm ⁻¹ FW)					
Sr. No.	Treatments	1DBS	1DAS	5 DAS	10DAS
1	Ametoctradin 27% SC + Dimethomorph 20.27% SC	0.07	0.14	0.16	0.12
2	Metalaxyl 8% + Mancozeb 64% WP	0.09	0.15	0.18	0.13
3	Cymoxil 8% + Mancozeb 64% WP	0.08	0.14	0.16	0.12
4	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC	0.08	0.12	0.14	0.11
5	Dimethomorph 50 WP	0.07	0.13	0.13	0.12
6	Metiram 55% + Pyraclostrobin 50% WG	0.06	0.14	0.15	0.11
7	Azoxystrobin 23 SC	0.08	0.10	0.13	0.01
8	Control (no spray)	0.07	0.08	0.07	0.08
S. Em. (±)		0.002	0.003	0.004	0.003
C. D. at 5%		0.01	0.01	0.01	0.01
C. V.%		4.56	4.57	4.61	4.56

Table 5: Effects of fungicidal treatment on the chlorophyll content

Chlorophyll content (mg/g fresh leaf)					
Sr. no.	Treatments	1DBS	1DAS	5DAS	10DAS
1	Ametoctradin 27% SC + Dimethomorph 20.27% SC	1.63	1.70	1.73	1.81
2	Metalaxyl 8% + Mancozeb 64% WP	1.65	1.71	1.75	1.83
3	Cymoxil 8% + Mancozeb 64% WP	1.67	1.70	1.74	1.82
4	Chlorothalonil 33.1% + Metalaxyl M 3.3% SC	1.66	1.60	1.73	1.79
5	Dimethomorph 50% WP	1.69	1.70	1.72	1.79
6	Metiram 55% + Pyraclostrobin 50% WG	1.68	1.70	1.74	1.80
7	Azoxystrobin 23% SC	1.65	1.69	1.72	1.76
8	Control (no spray)	1.64	1.64	1.53	1.43
S. Em. (±)		0.03	0.02	0.05	0.06
C. D. at 5%		0.09	0.06	0.13	0.14
C. V.%		4.71	4.63	4.54	4.55

**Fig 4:** Effect of fungicides on Peroxidase activity

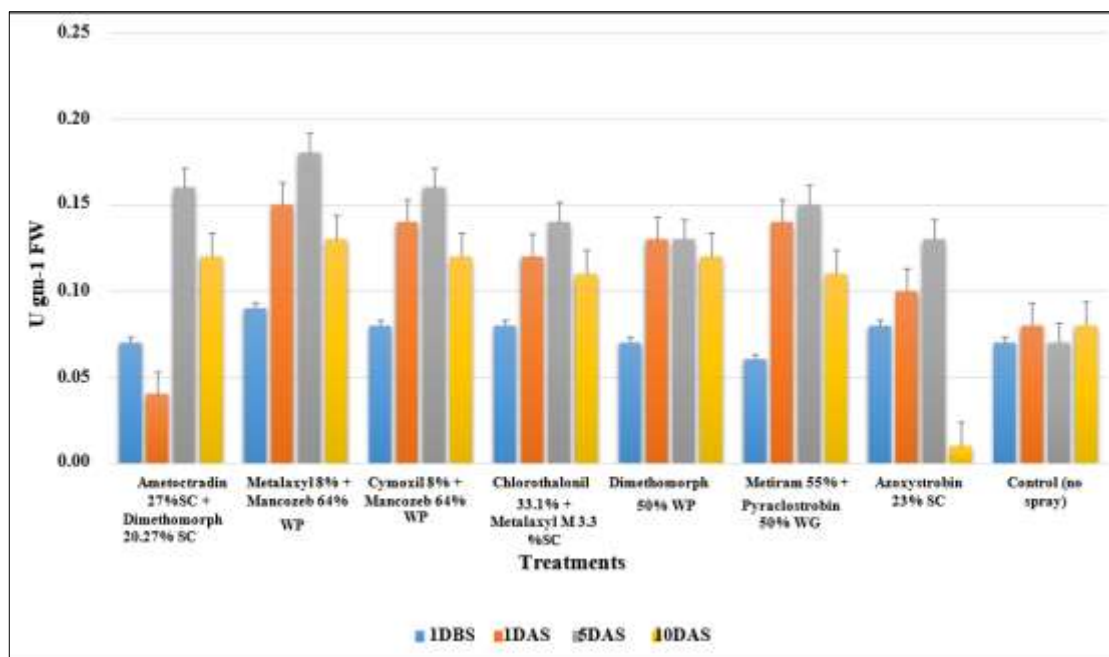


Fig 5: Effect of fungicides on Polyphenol oxidase activity

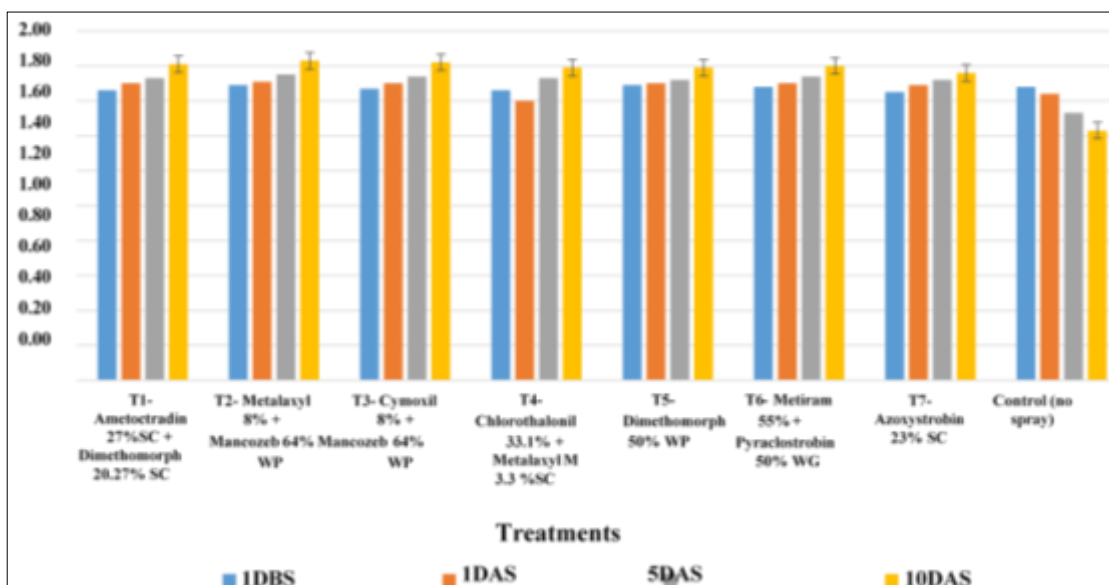


Fig 6: Effect of fungicides on Total Chlorophyll

Conclusion

This field study confirms novel fungicide combinations effectively manage cucumber downy mildew caused by *Pseudoperonospora cubensis*, with Metalaxyl 8% + Mancozeb 64% WP (0.2%) delivering superior results: lowest PDI (22.76%), AUDPC (380.80 units) and highest yield (153.43 q/ha, 191.14% increase over control). Treatments elevated peroxidase (0.18 U/g FW), polyphenol oxidase and chlorophyll (1.83 mg/g FW) levels, bolstering plant defence mechanisms 1-5 days post-application. These options mitigate resistance risks and phytotoxicity, providing sustainable control in humid North Gujarat climates.

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