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EFFICACY OF COMBINATION FUNGICIDES AGAINST DOWNY MILDEW AND ANTHRACNOSE IN GRAPES (*VITIS VINIFERA* L.)

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ABSTRACT

Grapes (*Vitis vinifera* L.) are a high-value fruit crop, but their productivity and marketability are constrained by fungal diseases, particularly downy mildew (*Plasmopara viticola*) and anthracnose (*Elsinoe ampelina*). This study evaluated the efficacy of selected combination fungicides under laboratory and field conditions during 2022–23 and 2023–24 at the Main Horticulture Research and Extension Centre, Bagalkot, Karnataka, India. *In vitro* assays using cavity slide and poisoned food techniques revealed that Azoxystrobin 25% + Tebuconazole 25% SC and Fluopyram 25% + Tebuconazole 25% WG completely inhibited sporangial germination of *P. viticola* and mycelial growth of *E. ampelina* at all tested concentrations. Based on laboratory performance, the most effective fungicides were evaluated in the field on eight-year-old grapevines *cv.* Thompson Seedless. Azoxystrobin 25% + Tebuconazole 25% SC and Tebuconazole 25% + Trifloxystrobin 25% WG recorded the lowest per cent disease index for both diseases, resulting in maximum disease reduction, highest yield, and superior net returns and benefit–cost ratio. These findings demonstrate that combination fungicides with multiple modes of action effectively manage grape fungal diseases, enhance yield, provide economic benefits and contribute to sustainable disease management in both domestic and export-oriented grape production systems.

Keywords: Grapes, Downy mildew, Anthracnose, Combination fungicides, Disease management, Yield, Economic analysis.

Introduction

Grapes (*Vitis vinifera* L.), belonging to the family Vitaceae, are among the most economically important fruit crops worldwide, valued for their versatility as fresh table grapes, raisins, juice and wine (Winkler *et al.*, 1974). In India, grapes were introduced from Iran and Afghanistan around the 14th century and gradually spread to south India in the 19th century (Randhawa and Mukherjee., 1986). Major grape growing states include Maharashtra, Karnataka, Tamil Nadu, and Andhra Pradesh, with Maharashtra alone contributing the largest share of production (APEDA, 2025). Grapevines thrive under subtropical climates with hot,

dry summers, adequate sunshine and well-drained soils with pH 6.5–7.5 (Shikhamany and Chadha, 1999). Adequate pruning, typically summer and monsoon pruning, regulates vegetative growth and improves air circulation, while balanced nutrient management ensures quality yield (Chadha, 2006). Grapes are predominantly used for fresh consumption (71%), followed by raisins (27%), juice (1.5%) and wine (0.5%) (APEDA, 2025). The export market, particularly to the Middle East and European countries, requires high quality, residue free produce, highlighting the importance of effective disease

management in maintaining both yield and marketability (NHB, 2025).

Despite its commercial importance, grape production is severely constrained by several fungal diseases, among which Downy mildew caused by *Plasmopara viticola* and Anthracnose caused by *Elsinoe ampelina* are the most destructive during rainy season. Downy mildew was first reported in Europe in the late 19th century and subsequently introduced into India in the early 20th century (Gessler *et al.*, 2011). The disease develops rapidly under cool and humid conditions and causes oil-spot lesions on leaves, leading to defoliation and poor bunch development (Kennelly *et al.*, 2005). Anthracnose, also known as bird's-eye rot, was reported in India by Butler in 1903 and is prevalent in warm and humid grape-growing regions (Pearson and Goheen, 1988). The disease affects leaves, shoots and berries causing sunken necrotic lesions that significantly reduce yield and market quality. Combined yield losses due to these diseases may range from 10 to 50 per cent, depending on varietal susceptibility and environmental conditions (Gadoury *et al.*, 2012). As no commercially cultivated grape variety in India possesses stable resistance to these pathogens, present disease management completely depends on fungicidal applications (Pearson and Goheen, 1988).

Extensive and repeated use of single site fungicides has resulted in fungicide resistance development and concerns related to pesticide residues, which pose serious challenges for export oriented grape production (Gisi *et al.*, 2000). In this context, combi fungicides, consisting of active ingredients with different modes of action, have gained importance due to their broader disease management spectrum, preventive and curative activity and ability to delay resistance development (FRAC, 2024). Therefore, the present investigation was undertaken to evaluate the *in vitro* efficacy of selected combi fungicides, assess their field performance against downy mildew and anthracnose based on per cent disease index (PDI), and determine their effect on yield, cost–benefit ratio under field conditions.

Materials and Methods

Laboratory experiments were conducted during 2022–23 at the Main Horticulture Research and Extension Centre, University of Horticultural Sciences, Bagalkot, Karnataka, India to evaluate the efficacy of

selected combi fungicides against the pathogens causing downy mildew and anthracnose diseases of grapes under *In vitro* conditions.

Freshly infected grape leaves showing typical downy mildew symptoms, characterized by downy growth on the lower surface (Fig. 1) and corresponding yellow spots on the upper surface (Fig. 2), were collected from the vineyard. In severe cases, the entire leaf surface was covered with downy growth. Infected bunches showing downy growth (Fig. 3) and green twigs exhibiting large black lesions with downy growth were also collected (Fig. 4) (Mugnai *et al.*, 1999; Caffi *et al.*, 2016). The pathogen *P. viticola* was identified based on the presence of characteristic lemon-shaped sporangia borne on dichotomously branched sporangiophores with right-angled branching (Fig.5), as described by Gessler *et al.* (2011). Sporangial suspensions were prepared by gently washing the abaxial surface of infected leaves with sterile distilled water, followed by filtration through muslin cloth. The spore concentration was adjusted using a haemocytometer. Fungicidal efficacy was assessed using the cavity slide sporangial germination assay, wherein one drop (1 ml) of sporangial suspension was mixed with an equal volume of fungicide solution prepared at 1/10, 1/7.5 and 1/5 of the recommended field dose (RDF) (Tables 1 and 2). Slides containing sporangial suspension mixed with sterile distilled water served as the untreated control. The slides were incubated in a moist chamber at 25 ± 1 °C for 24 h. After incubation, sporangial germination was examined under a compound microscope at 40× magnification and at least 100 sporangia per slide were observed. Sporangia showing germ tube emergence were considered germinated and per cent inhibition over control was calculated (Chavan *et al.*, 2021; Gadoury *et al.*, 2012). Each treatment was replicated thrice and the experiment was repeated to ensure reproducibility.

Freshly infected grape leaves showing typical anthracnose symptoms, characterized by circular to irregular dark brown sunken lesions with grey centres and black margins (Fig. 6), along with infected green twigs exhibiting elongated cankers (Fig. 7) and affected bunches with dark necrotic spots, were collected from the vineyard following standard symptom descriptions (Sawant *et al.*, 2012; Patil *et al.*, 2018; Chatta, 1992; Sood *et al.*, 2020).



Fig.1. Downy growth on lower surface of leaf



Fig.2. Yellow spots on upper surface of leaf



Fig.3. Downy growth on bunch



Fig.4. Downy mildew infection on twigs



Fig.5. Lemon-shaped sporangia of *Plasmopara viticola* borne on dichotomously branched (40X)

Table 1: Evaluation of combi fungicides against downy mildew and anthracnose disease in grapes

Tr. No.	Combi Fungicide (Active ingredients)	FRAC Code	General Mode of Action	Specific Cellular / Biochemical Mode of Action	Dose / ha	Dose / litre
T1	Azoxystrobin 25% + Tebuconazole 25% SC	11 + 3	Systemic, preventive & curative	Azoxystrobin: inhibits mitochondrial electron transport at the Qo site, blocking ATP synthesis. Tebuconazole: inhibits C-14 demethylation in ergosterol biosynthesis, disrupting cell membrane formation.	375 ml	0.5–0.75 ml
T2	Fluopyram 25% + Tebuconazole 25% WG	7 + 3	Systemic, broad spectrum	Fluopyram: inhibits succinate dehydrogenase (SDH) in mitochondrial complex II, reducing energy production. Tebuconazole: inhibits ergosterol biosynthesis affecting membrane integrity.	375 g	0.5 g
T3	Boscalid 25% + Pyraclostrobin 25% WG	7 + 11	Systemic, preventive	Boscalid: blocks succinate dehydrogenase in mitochondrial respiration. Pyraclostrobin: inhibits electron transfer at the Qo site, leading to ATP depletion.	500 g	0.75–1.0 g
T4	Tricyclazole 18% + Mancozeb 62% WP	16 + M3	Systemic + contact (protective)	Tricyclazole: inhibits melanin biosynthesis required for functional appressoria and host penetration. Mancozeb: multi-site enzyme inhibitor acting on sulphhydryl (–SH) groups, disrupting cellular metabolism.	300 g	0.5 g
T5	Mandipropamid 9% + Difenconazole 6% SC	40 + 3	Translaminar + systemic	Mandipropamid: inhibits cellulose synthase involved in cell wall formation of oomycetes. Difenconazole: inhibits sterol biosynthesis by blocking C-14 demethylation.	300 ml	0.5 ml
T6	Cymoxanil 8% + Mancozeb 64% WP	27 + M3	Local systemic + contact	Cymoxanil: disrupts nucleic acid and protein synthesis during early infection stages. Mancozeb: inhibits multiple cytoplasmic enzyme systems.	2500 g	2.0 g
T7	Benalaxyl-M 4% + Mancozeb 64% WP	4 + M3	Systemic + protective	Benalaxyl-M: inhibits RNA synthesis in oomycetes, affecting mycelial growth. Mancozeb: multi-site inhibitor of enzymatic metabolic processes.	2500 g	2.5 g
T8	Tebuconazole 25% + Trifloxystrobin 25% WG	3 + 11	Systemic, preventive & curative	Tebuconazole: inhibits ergosterol biosynthesis required for fungal cell membranes. Trifloxystrobin: blocks mitochondrial respiration at the Qo site.	375 g	0.5 g
T9	Copper oxychloride 50% + Mancozeb 25% WP	M1 + M3	Contact, multi-site	Copper oxychloride: releases Cu ²⁺ ions that denature proteins and enzymes. Mancozeb: inhibits multiple enzyme systems through sulphhydryl binding.	3000 g	3.0 g
T10	Difenconazole 25% + Azoxystrobin 25% SC	3 + 11	Systemic, preventive & curative	Difenconazole: inhibits sterol biosynthesis essential for membrane development. Azoxystrobin: inhibits mitochondrial electron transport at the Qo site.	375 ml	0.5–0.75 ml

Table 2: *In vitro* evaluation of combi fungicides at different dosage

Tr. No.	Combi Fungicide	Formulation	Field Dose	C1 (1/10 FD)	C2 (1/7.5 FD)	C3 (1/5 FD)
T1	Azoxystrobin 25% + Tebuconazole 25% SC	SC	0.75 ml/lit	0.075 ml/lit	0.10 ml/lit	0.15 ml/lit
T2	Fluopyram 25% + Tebuconazole 25% WG	WG	0.5 g/lit	0.05 g/lit	0.067 g/lit	0.10 g/lit
T3	Boscalid 25% + Pyraclostrobin 25% WG	WG	1.0 g/lit	0.10 g/lit	0.133 g/lit	0.20 g/lit
T4	Tricyclazole 18% + Mancozeb 62% WP	WP	0.5 g/lit	0.05 g/lit	0.067 g/lit	0.10 g/lit
T5	Mandipropamid 9% + Difenconazole 6% SC	SC	0.5 ml/lit	0.05 ml/lit	0.067 ml/lit	0.10 ml/lit
T6	Cymoxanil 8% + Mancozeb 64% WP	WP	2.0 g/lit	0.20 g/lit	0.267 g/lit	0.40 g/lit
T7	Benalaxyl-M 4% + Mancozeb 64% WP	WP	2.5 g/lit	0.25 g/lit	0.333 g/lit	0.50 g/lit
T8	Tebuconazole 25% + Trifloxystrobin 25% WG	WG	0.5 g/lit	0.05 g/lit	0.067 g/lit	0.10 g/lit
T9	Copper oxychloride 50% + Mancozeb 25% WP	WP	3.0 g/lit	0.30 g/lit	0.40 g/lit	0.60 g/lit
T10	Difenconazole 25% + Azoxystrobin 25% SC	SC	00.75 ml/lit	0.075 ml/lit	0.10 ml/lit	0.15 ml/lit

C1 = 1/5 Field dose; C2 = 1/10 Field dose; C3 = 1/15 Field dose; FD- Field dose

The pathogen was identified based on the presence of septate mycelium with branched conidiophores bearing cylindrical to oblong conidia

(Fig.8), as described by Gouse *et al.* (2025) and Chatta (1992). Spore suspensions were prepared by gently scraping the surface of infected leaves, twigs, and

lesions, followed by filtration through sterile muslin cloth. The spore concentration was adjusted using a haemocytometer. Fungicidal efficacy was evaluated using the poisoned food technique. Potato dextrose agar (PDA) medium (100 ml) was prepared in 250 ml conical flasks and molten PDA was amended with the required quantity of fungicide to obtain concentrations corresponding to 1/5, 1/10 and 1/15 of the recommended field dose (Tables 1 and 2). The amended medium was mixed thoroughly under aseptic conditions inside a laminar airflow cabinet and poured into 90 mm sterile Petri plates. Unamended PDA served as the control. A 5 mm mycelial disc taken from the actively growing margin of a pure culture of *E. ampelina* was placed at the centre of each plate. Plates were incubated at 25 ± 1 °C until full fungal growth was attained in the untreated control (Gouse *et al.*, 2025). Radial mycelial growth was measured in two perpendicular directions, and percent inhibition over control was calculated as described by Kumar *et al.* (2022). Treatments were replicated thrice, and

fungicides showing superior inhibition under laboratory conditions were selected for subsequent field evaluation.

Field experiments were conducted during two consecutive seasons (2022–23 and 2023–24) at MHREC, Bagalkot, on eight year old grapevines *cv.* Thompson Seedless, trained on the “Y” system and spaced at 10 × 5 feet in red loamy soil. The experiment consisted of seven treatments, including an untreated control, laid out in a randomized block design with three replications. Each treatment comprised 12 vines per replication and five vines were tagged for recording observations. Based on laboratory screening, selected fungicides were applied after the onset of disease symptoms following fruit pruning during October–November, using a knapsack sprayer with a spray volume of 1000 lit/ha. Two sprays were given at a 10 days interval. Disease observations were recorded one day before the first spray, 10 days after the first spray, and 10 days after the second spray.



Fig.6. Anthracnose disease symptoms on leaves



Fig.7. Anthracnose disease symptoms on twigs

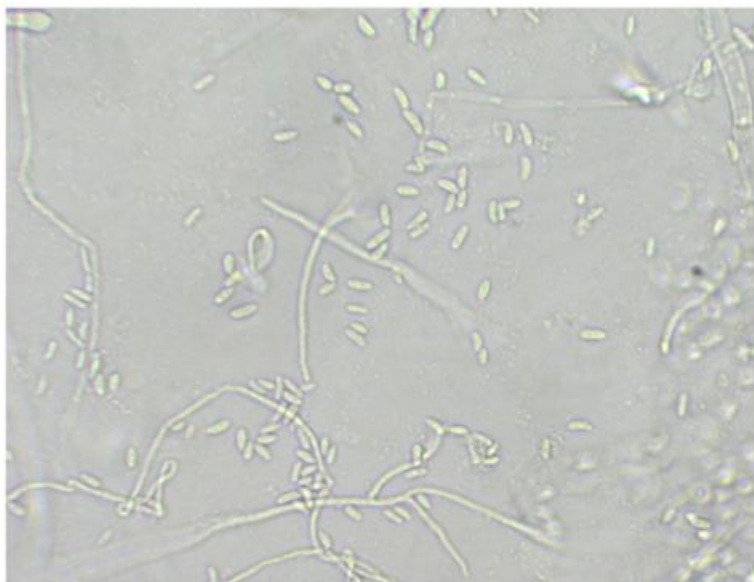


Fig. 8. Septate mycelium with branched conidiophores bearing cylindrical to oblong conidia of grape anthracnose pathogen *Elsinoe ampelina* (40X)

Downy mildew severity on grape leaves and bunches was assessed using the disease rating scale of Horsfall and Barratt (1986), in which five disease categories were made based on the percentage of leaf area infected: 0 = no visible symptoms, 1 = trace to 25 per cent leaf area infected, 2 = 26–50 per cent leaf area infected, 3 = 51–75 per cent leaf area infected, and 4 = more than 75 per cent leaf area infected. Anthracnose disease severity was scored using the 0–4 scale given by Chatta (1992), where 0 indicated healthy foliage or leaf spots in traces, 1 represented up to 10 per cent leaf area covered with anthracnose lesions, 2 indicated 10.1–25 per cent leaf area covered with slight twig infection (1–3 cankers per twig), 3 represented 25.1–50 per cent leaf area covered with heavy twig infection (4–10 cankers per twig), and 4 indicated above 50 per cent leaf area covered with very heavy twig infection (more than 10 cankers per twig) along with heavy berry infection. The disease severity data were converted into Per cent Disease Index (PDI) using the formula given by Wheeler (1969).

$$\text{PDI} = \frac{\sum \text{of all numerical ratings}}{\text{Total number of observations}} \times 100$$

× Maximum disease rating

Yield was recorded as yield per vine (kg) from tagged vines and converted to yield per hectare (tons/ha). Economic analysis was performed by calculating

gross returns based on the prevailing local APMC market price, followed by computation of net returns and benefit–cost (B:C) ratio using standard procedures outlined by Ghosh *et al.* (2019). All data were subjected to statistical analysis using SPSS software, and treatment means were compared at $P = 0.05$.

Results

The *in vitro* evaluation of combi fungicides against *P. viticola* showed significant variation in sporangial germination inhibition across treatments and concentrations (Table 3). Azoxystrobin 25% + Tebuconazole 25% SC (Fig.9) and Fluopyram 25% + Tebuconazole 25% WG consistently recorded complete inhibition (100%) at all tested concentrations and it indicating excellent anti-sporulant activity. Boscalid 25% + Pyraclostrobin 25% WG and Tebuconazole 25% + Trifloxystrobin 25% WG were statistically on par, maintaining more than 94 per cent inhibition even at the lowest dose. Mandipropamid 9% + Difenconazole 6% SC, Cymoxanil 8% + Mancozeb 64% WP and Benalaxyl-M 4% + Mancozeb 64% WP exhibited moderate dose-dependent inhibition. Tricyclazole 18% + Mancozeb 62% WP, Difenconazole 25% + Azoxystrobin 25% SC and Copper oxychloride 50% + Mancozeb 25% WP were comparatively less effective, while no inhibition was observed in the untreated control.

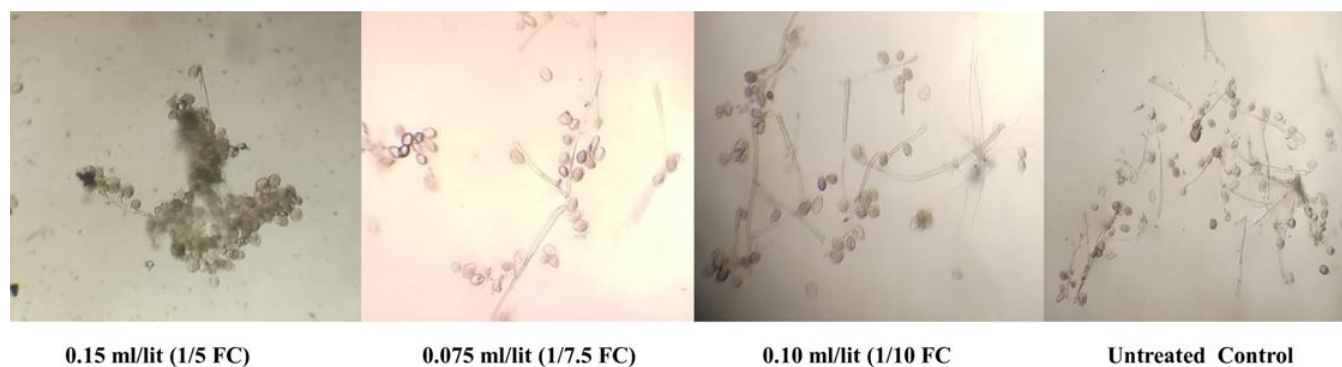


Fig. 9. *In vitro* efficacy of Azoxystrobin 25% + Tebuconazole 25% SC against *Plasmopara viticola*

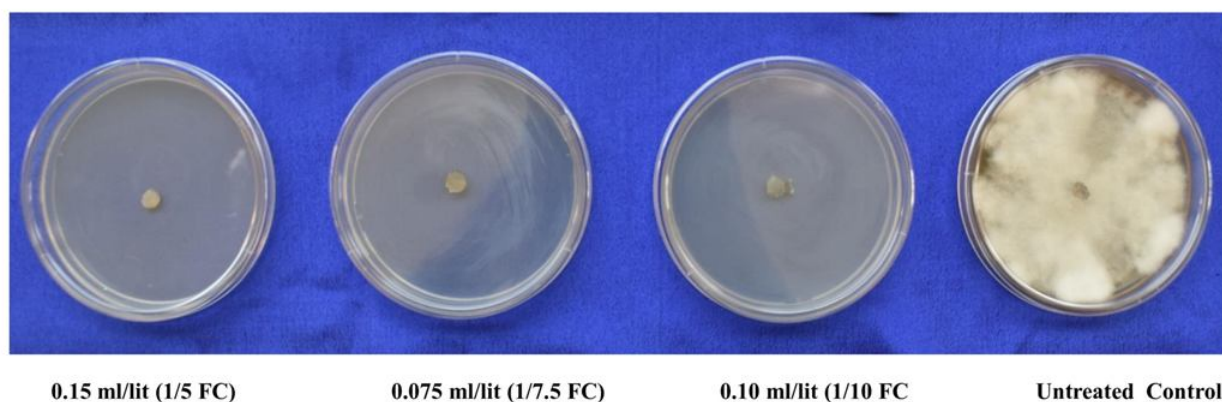


Fig. 10. Inhibition of mycelial growth of *Elsinoe ampelina* by Azoxystrobin 25% + Tebuconazole 25% SC under *in vitro* condition

Against *E. ampelina*, most of the combi fungicides exhibited strong fungitoxic effects, with clear differences among tested treatments (Table 4). Azoxystrobin 25% + Tebuconazole 25% SC (Fig.10), Fluopyram 25% + Tebuconazole 25% WG and Tebuconazole 25% + Trifloxystrobin 25% WG resulted in near complete to complete inhibition of mycelial growth at all tested concentrations. Boscalid 25% + Pyraclostrobin 25% WG also showed consistently high efficacy, with inhibition exceeding 93 per cent even at reduced doses. Moderate inhibition was recorded with Difenconazole 25% + Azoxystrobin 25% SC and Tricyclazole 18% + Mancozeb 62% WP, whereas Cymoxanil 8% + Mancozeb 64% WP, Benalaxyl-M 4% + Mancozeb 64% WP, Mandipropamid 9% + Difenconazole 6% SC and Copper oxychloride 50% + Mancozeb 25% WP were less effective. The untreated control showed unrestricted mycelial growth.

Field evaluation of combi fungicides against downy mildew disease of grapes during 2022–23 and 2023–24 revealed significant differences among

treatments (Tables 5). Tebuconazole 25% + Trifloxystrobin 25% WG consistently recorded the lowest PDI on leaves (10.80% and 10.00%) and bunches (14.20% and 13.50%) after the second spray, resulting in the highest disease reduction of 81.00–81.60 per cent on leaves and 77.20–77.40 per cent on bunches. Azoxystrobin 25% + Tebuconazole 25% SC and Boscalid 25% + Pyraclostrobin 25% WG were statistically on par, registering PDI values of 11.20–12.40 per cent on leaves and 14.60–15.80 per cent on bunches, with disease reduction ranging from 76.60 to 80.80 per cent. Fluopyram 25% + Tebuconazole 25% WG also provided effective disease management with 73.70–77.50 per cent disease reduction. Mandipropamid 9% + Difenconazole 6% SC and Tricyclazole 18% + Mancozeb 62% WP were comparatively less effective. In contrast, the untreated control recorded the highest disease severity with PDI values of 56.80 per cent and 54.20 per cent on leaves and 62.40 per cent and 59.80 per cent on bunches during 2022–23 and 2023–24, respectively.

Table 3: *In vitro* evaluation of combi fungicides against *Plasmopara viticola*

Tr. No.	Combi Fungicide	C1 (1/5 FD)	C2 (1/7.5 FD)	C3 (1/10FD)
T1	Azoxystrobin 25% + Tebuconazole 25% SC	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a
T2	Fluopyram 25% + Tebuconazole 25% WG	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a
T3	Boscalid 25% + Pyraclostrobin 25% WG	100.00 ± 0.00 ^a	99.35 ± 0.89 ^a	98.42 ± 1.27 ^a
T4	Tebuconazole 25% + Trifloxystrobin 25% WG	98.76 ± 1.12 ^a	96.38 ± 1.54 ^a	94.25 ± 1.86 ^a
T5	Mandipropamid 9% + Difenconazole 6% SC	82.45 ± 2.04 ^b	76.88 ± 2.31 ^b	70.26 ± 2.64 ^b
T6	Cymoxanil 8% + Mancozeb 64% WP	78.34 ± 2.28 ^b	71.62 ± 2.55 ^b	64.85 ± 2.78 ^b
T7	Benalaxyl-M 4% + Mancozeb 64% WP	73.18 ± 2.46 ^b	66.52 ± 2.71 ^b	59.44 ± 2.94 ^c
T8	Tricyclazole 18% + Mancozeb 62% WP	58.64 ± 2.82 ^c	50.26 ± 3.04 ^c	42.35 ± 3.26 ^c
T9	Difenconazole 25% + Azoxystrobin 25% SC	55.78 ± 2.69 ^c	47.42 ± 2.96 ^c	39.86 ± 3.18 ^c
T10	Copper oxychloride 50% + Mancozeb 25% WP	51.32 ± 2.91 ^c	43.18 ± 3.12 ^c	35.74 ± 3.35 ^c
T11	Untreated control	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d

Table 4: *In vitro* evaluation of combi fungicides against *Elsinoe ampelina*

Tr. No.	Combi Fungicide	C1 (1/5 FD)	C2 (1/7.5FD)	C3 (1/10 FD)
T ₁	Azoxystrobin 25% + Tebuconazole 25% SC	100.00 ± 0.00 ^a	98.65 ± 0.92 ^a	96.48 ± 1.36 ^a
T ₂	Fluopyram 25% + Tebuconazole 25% WG	100.00 ± 0.00 ^a	99.18 ± 0.74 ^a	97.24 ± 1.15 ^a
T ₃	Boscalid 25% + Pyraclostrobin 25% WG	98.74 ± 1.08 ^a	96.42 ± 1.46 ^a	93.85 ± 1.88 ^a
T ₄	Tebuconazole 25% + Trifloxystrobin 25% WG	100.00 ± 0.00 ^a	99.46 ± 0.68 ^a	97.88 ± 1.12 ^a
T ₅	Difenconazole 25% + Azoxystrobin 25% SC	89.62 ± 1.84 ^b	84.36 ± 2.12 ^b	78.24 ± 2.48 ^b
T ₆	Tricyclazole 18% + Mancozeb 62% WP	82.74 ± 2.26 ^b	76.18 ± 2.54 ^b	69.32 ± 2.81 ^b
T ₇	Cymoxanil 8% + Mancozeb 64% WP	68.36 ± 2.74 ^c	60.42 ± 2.98 ^c	52.88 ± 3.16 ^c
T ₈	Benalaxyl-M 4% + Mancozeb 64% WP	64.28 ± 2.88 ^c	56.64 ± 3.04 ^c	48.72 ± 3.26 ^c
T ₉	Mandipropamid 9% + Difenconazole 6% SC	61.54 ± 2.96 ^c	53.18 ± 3.14 ^c	45.26 ± 3.38 ^c
T ₁₀	Copper oxychloride 50% + Mancozeb 25% WP	58.32 ± 3.02 ^c	49.86 ± 3.28 ^c	41.18 ± 3.54 ^c
T ₁₁	Untreated control	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d

A similar trend was observed for anthracnose management (Table 6). Tebuconazole 25% + Trifloxystrobin 25% WG proved most effective, recording the lowest PDI on leaves (11.80% and 11.20%) and twigs (14.20% and 13.40%), corresponding to disease reduction of 79.10–79.30 per cent on leaves and 76.90–77.30 per cent on twigs. Azoxystrobin 25% + Tebuconazole 25% SC, Boscalid 25% + Pyraclostrobin 25% WG and Fluopyram 25% + Tebuconazole 25% WG were statistically comparable, with disease reduction ranging from 73.00 to 78.30 per cent. Mandipropamid 9% + Difenconazole 6% SC and Tricyclazole 18% + Mancozeb 62% WP showed moderate control. The untreated control exhibited maximum disease intensity, recording PDI values of 56.80 per cent and 54.20 per cent on leaves and 62.40 per cent and 59.80 per cent on twigs during 2022–23 and 2023–24, respectively.

Yield data clearly reflected the effectiveness of disease management practices (Table 7). The highest mean yield (23.80 tons/ ha) was obtained with Tebuconazole 25% + Trifloxystrobin 25% WG, followed by Azoxystrobin 25% + Tebuconazole 25%

SC (23.30 tons/ ha) and Fluopyram 25% + Tebuconazole 25% WG (22.70 tons/ ha). Boscalid 25% + Pyraclostrobin 25% WG also recorded higher yields (22.30 tons/ ha) compared to the untreated control, which produced only 3.50 tons/ ha. Moderate yields were obtained with Mandipropamid 9% + Difenconazole 6% SC (17.80 tons/ ha) and Tricyclazole 18% + Mancozeb 62% WP (16.70 tons/ ha).

Economic analysis further substantiated these findings (Table 8). Tebuconazole 25% + Trifloxystrobin 25% WG recorded the highest net return (Rs. 5,01,400/ ha) with a superior benefit–cost ratio (1.93:1), followed by Azoxystrobin 25% + Tebuconazole 25% SC (Rs. 4,85,600/ ha; B:C ratio 1.87:1). Other effective treatments also recorded favourable returns, whereas Mandipropamid 9% + Difenconazole 6% SC and Tricyclazole 18% + Mancozeb 62% WP resulted in comparatively lower profitability. The untreated control showed the lowest economic returns with a B:C ratio of 0.56:1, clearly demonstrating the economic advantage of effective combi fungicide application under field conditions.

Table 5: Field evaluation of combi fungicides against downy mildew disease of grapes

Tr. No.	Treatments	Dosage	2022-23				2023-24			
			PDI on leaves after 2 nd spray	Per cent disease reduction over control	PDI on bunch after 2 nd spray	Per cent disease reduction over control	PDI on leaves after 2 nd spray	Per cent disease reduction over control	PDI on bunch after 2 nd spray	Per cent disease reduction over control
T ₁	Azoxystrobin 25% + Tebuconazole 25% SC	375 ml/ha	11.20 ± 1.00 ^a	80.30	14.60 ± 1.30 ^a	76.60	10.40 ± 0.90 ^a	80.80	13.80 ± 1.10 ^a	76.90
T ₂	Fluopyram 25% + Tebuconazole 25% WG	375 g/ha	13.00 ± 1.20 ^a	77.10	16.40 ± 1.50 ^a	73.70	12.20 ± 1.10 ^a	77.50	15.20 ± 1.30 ^a	74.60
T ₃	Boscalid 25% + Pyraclostrobin 25% WG	500 g/ha	12.40 ± 1.10 ^a	78.20	15.80 ± 1.40 ^a	74.70	11.80 ± 1.00 ^a	78.20	14.90 ± 1.20 ^a	75.10
T ₄	Tebuconazole 25% + Trifloxystrobin 25% WG	375 g/ha	10.80 ± 0.90 ^a	81.00	14.20 ± 1.10 ^a	77.20	10.00 ± 0.80 ^a	81.60	13.50 ± 1.00 ^a	77.40
T ₅	Mandipropamid 9% + Difenconazole 6% SC	300 ml/ha	17.60 ± 1.50 ^b	69.00	21.40 ± 1.90 ^b	65.70	16.80 ± 1.30 ^b	69.00	20.60 ± 1.70 ^b	65.60
T ₆	Tricyclazole 18% + Mancozeb 62% WP	300 g/ha	20.80 ± 1.80 ^c	63.40	25.60 ± 2.10 ^c	59.00	19.80 ± 1.70 ^c	63.50	24.80 ± 2.00 ^c	58.60
T ₇	Untreated Control	—	56.80 ± 3.20 ^d	—	62.40 ± 2.80 ^d	—	54.20 ± 3.00 ^d	—	59.80 ± 2.60 ^d	—

Table 6: Field evaluation of combi fungicides against anthracnose disease of grapes

Tr. No.	Treatments	Dosage	2022-23				2023-24			
			PDI on leaves after 2 nd spray	Per cent disease reduction over control	PDI on twigs after 2 nd spray	Per cent disease reduction over control	PDI on leaves after 2 nd spray	Per cent disease reduction over control	PDI on twigs after 2 nd spray	Per cent disease reduction over control
T ₁	Azoxystrobin 25% + Tebuconazole 25% SC	375 ml/ha	12.40 ± 1.10 ^a	78.20	14.60 ± 1.20 ^a	76.50	11.80 ± 1.00 ^a	78.30	13.80 ± 1.10 ^a	76.80
T ₂	Fluopyram 25% + Tebuconazole 25% WG	375 g/ha	14.20 ± 1.30 ^a	75.10	16.40 ± 1.40 ^a	73.00	13.00 ± 1.20 ^a	75.50	15.20 ± 1.30 ^a	73.50
T ₃	Boscalid 25% + Pyraclostrobin 25% WG	500 g/ha	13.60 ± 1.20 ^a	76.40	15.80 ± 1.30 ^a	74.90	12.80 ± 1.10 ^a	76.30	14.90 ± 1.20 ^a	75.20
T ₄	Tebuconazole 25% + Trifloxystrobin 25% WG	375 g/ha	11.80 ± 0.90 ^a	79.10	14.20 ± 1.10 ^a	77.30	11.20 ± 0.90 ^a	79.30	13.40 ± 1.00 ^a	76.90
T ₅	Mandipropamid 9% + Difenconazole 6% SC	300 ml/ha	21.40 ± 1.60 ^c	62.40	25.60 ± 1.80 ^c	59.20	20.80 ± 1.50 ^c	61.60	24.80 ± 1.70 ^c	58.60
T ₆	Tricyclazole 18% + Mancozeb 62% WP	300 g/ha	18.20 ± 1.40 ^b	68.20	21.40 ± 1.60 ^b	65.80	17.40 ± 1.30 ^b	68.00	20.60 ± 1.50 ^b	65.80
T ₇	Untreated Control	—	56.80 ± 3.20 ^d	—	62.40 ± 3.00 ^d	—	54.20 ± 3.00 ^d	—	59.80 ± 2.80 ^d	—

Table 7: Effect of combi fungicides on yield of grapes

Tr. No.	Treatments	Dosage	2022-23		2023-24		Mean Yield (tons)
			Yield/ vein (Kg)	Yield/ha (tons)	Yield/ vein (Kg)	Yield/ha (tons)	
T ₁	Azoxystrobin 25% + Tebuconazole 25% SC	375 ml/ha	16.50 ± 0.80	23.00	16.80 ± 0.85	23.60	23.30
T ₂	Fluopyram 25% + Tebuconazole 25% WG	375 g/ha	16.20 ± 0.75	22.40	16.50 ± 0.80	23.00	22.70
T ₃	Boscalid 25% + Pyraclostrobin 25% WG	500 g/ha	16.00 ± 0.80	22.00	16.30 ± 0.80	22.60	22.30
T ₄	Tebuconazole 25% + Trifloxystrobin 25% WG	375 g/ha	17.00 ± 0.85	24.00	16.80 ± 0.80	23.60	23.80
T ₅	Mandipropamid 9% + Difenconazole 6% SC	300 ml/ha	14.00 ± 0.70	18.00	13.80 ± 0.75	17.60	17.80
T ₆	Tricyclazole 18% + Mancozeb 62% WP	300 g/ha	13.20 ± 0.70	16.40	13.50 ± 0.70	17.00	16.70
T ₇	Untreated Control	—	1.80 ± 0.25	3.60	1.70 ± 0.20	3.40	3.50

Table 8: Cost–benefit analysis of combi fungicides for grapes

Tr. No.	Treatments	Dosage	Chemical cost (Rs./ha, 2 sprays)	Cultivation cost (Rs./ha)	Total cost (Rs./ha)	Mean yield (tons/ha)	Incremental yield (tons/ha)	Gross return (Rs./ha)	Net return (Rs./ha)	CBR
T1	Azoxystrobin 25% + Tebuconazole 25% SC	375 ml/ha	6,000	2,54,000	2,60,000	23.30	19.80	7,45,600	4,85,600	1.87
T2	Fluopyram 25% + Tebuconazole 25% WG	375 g/ha	5,500	2,54,000	2,59,500	22.70	19.20	7,26,400	4,66,900	1.80
T3	Boscalid 25% + Pyraclostrobin 25% WG	500 g/ha	6,500	2,54,000	2,60,500	22.30	18.80	7,13,600	4,53,100	1.74
T4	Tebuconazole 25% + Trifloxystrobin 25% WG	375 g/ha	6,200	2,54,000	2,60,200	23.80	20.30	7,61,600	5,01,400	1.93
T5	Mandipropamid 9% + Difenconazole 6% SC	300 ml/ha	5,800	2,54,000	2,59,800	17.80	14.30	5,69,600	3,09,800	1.19
T6	Tricyclazole 18% + Mancozeb 62% WP	300 g/ha	5,500	2,54,000	2,59,500	16.70	13.20	5,34,400	2,74,900	1.06
T7	Untreated Control	—	0.00	2,54,000	2,54,000	3.50	—	1,12,000	-1,42,000	0.56

Fresh grapes price at APMC Vijayapur: Rs- 32,000/tons; CBR- Benefit cost ratio

Discussion

The present study clearly established the superiority of selected combi fungicides against downy mildew (*P. viticola*) and anthracnose (*E. ampelina*) of grapes under both laboratory and field conditions. Complete inhibition of sporangial germination by Azoxystrobin 25% + Tebuconazole 25% SC and Fluopyram 25% + Tebuconazole 25% WG at all *in vitro* concentrations highlights the advantage of combining fungicides with different biochemical targets. The combined action of QoI (strobilurin) and DMI (triazole) fungicides disrupts mitochondrial respiration and sterol biosynthesis simultaneously, leading to rapid pathogen suppression and reduced disease incidence. Similar synergistic benefits of QoI–DMI mixtures against downy mildew and other oomycetes have been reported in high-impact journals (Gisi *et al.*, 2000; Stewart *et al.*, 2008; Latorre and Doster., 2018).

Azoxystrobin, trifloxystrobin and boscalid act by inhibiting mitochondrial electron transport and SDH activity, while tebuconazole and difenoconazole inhibit ergosterol biosynthesis, affecting membrane formation and cell viability. These modes of action have been validated in grape disease studies from both Indian and international contexts (Caffi *et al.*, 2016; Bove *et al.*, 2020). High efficacy of Boscalid + Pyraclostrobin and Tebuconazole + Trifloxystrobin mixtures in this investigation supports earlier observations that SDHI–QoI and triazole–QoI combinations significantly reduce sporulation and disease progression even at reduced doses (Bartlett *et al.*, 2002; Gisi and Sierotzki., 2008).

A comparable response was observed against *E. ampelina*, where triazole and strobilurin-based combi

fungicides produced near complete inhibition of mycelial growth. These results are in agreement with anthracnose management studies in grapes and other fruit crops showing high sensitivity of anthracnose pathogens to sterol biosynthesis inhibitors and respiration inhibitors (Sood *et al.*, 2020; Sharma *et al.*, 2019). Mancozeb-based and older multi-site chemistries, while effective as protectants, exhibited lower efficacy due to limited systemic and curative action, consistent with reports that contact fungicides alone may be insufficient in managing rapidly progressing infections (Crouch *et al.*, 2010).

Field performance closely mirrored laboratory results, validating *In vitro* screening as a robust approach for fungicide selection. Tebuconazole + Trifloxystrobin consistently recorded the lowest PDI on leaves, bunches and twigs for both diseases across seasons due to its combined preventive and curative properties. Reported field performance of QoI–DMI mixtures in managing downy mildew and botrytis bunch rot further corroborates these findings (Calonnet *et al.*, 2004; Rane *et al.*, 2012). Reduced efficacy of Mandipropamid and Mancozeb-based treatments under field conditions may be attributed to their narrower spectrum and limited post-infection activity, as also observed in comparative fungicide trials in Indian vineyards (Patil *et al.*, 2013; Kokate *et al.*, 2019).

Higher yields obtained with effective combi fungicides reflected improved disease suppression, leading to better canopy health, photosynthetic efficiency, and bunch development. Yield gains following efficient downy mildew control have been reported in several studies, reinforcing the agronomic and economic importance of integrated disease management (Ghosh *et al.*, 2019; Dhillon *et al.*, 2021).

Economic analysis further highlighted the advantage of combi fungicides, with Tebuconazole + Trifloxystrobin recording highest net returns and benefit–cost ratio, illustrating that effective disease control directly translates into improved profitability for growers.

Notably, strategic use of combi fungicides with multiple modes of action aligns with resistance management principles advocated by the Fungicide Resistance Action Committee (FRAC., 2024) and supported by global grape pathology research (Rotondo *et al.*, 2019; Stewart *et al.*, 2020). Alternating or combining modes of action reduces selective pressure on single biochemical targets, delaying resistance emergence a critical consideration given documented QoI resistance in downy mildew populations in several grape growing regions (Gisi *et al.*, 2000).

Conclusion

The present investigation demonstrated that combi fungicides with multiple modes of action provide highly effective and consistent management of downy mildew and anthracnose in grapes under both laboratory and field conditions. Among the tested treatments, Tebuconazole 25% + Trifloxystrobin 25% WG proved superior, recording the lowest disease severity, highest yield and maximum economic returns across seasons. The strong agreement between *in vitro* and field performance confirms the reliability of laboratory screening for selecting effective fungicides. Adoption of such broad-spectrum, resistance-managing combinations can significantly enhance productivity, fruit quality, and profitability in export-oriented grape production systems. Hence, these combi fungicides can be recommended as a key component of sustainable grape disease management strategies.

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