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## Performance and Phytotoxicity assessment of Mancozeb 40% + Azoxystrobin 7% OS against downy mildew of grapes in Maharashtra, India

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Grape (*Vitis vinifera*) is a commercially important fruit crop of India. Downy mildew (c.o. *Plasmopara viticola*) is a devastating fungal disease of grapes causing 60-70% of crop loss thereby altering the grape economy significantly. Mancozeb, an ethylene-bis-dithiocarbamate fungicide is extensively used to control a wide variety of plant diseases. Due to its multisite mode of action, mancozeb has not developed resistance to fungal diseases. Azoxystrobin, a systemic, broad-spectrum, Quinone outside Inhibitor fungicide exerts its fungicidal activity by inhibiting mitochondrial respiration in fungi. The combination of fungicide i.e. Mancozeb 40% + Azoxystrobin 7% OS was evaluated for two seasons under field conditions at Maharashtra. Different concentrations of Mancozeb 40% + Azoxystrobin 7% OS at viz. 1250, 1500 and 1750 g/ha were compared with its component fungicides Azoxystrobin 23%SC and Mancozeb 75% separately. Pooled data revealed that Mancozeb 40% + Azoxystrobin 7% OS @ 1750g/ha manifested lowest PDI of 21.94 with a corresponding yield of 24.9 t/ha. As Mancozeb 40% + Azoxystrobin 7% OS @1750 also was at par with dose of @1500g/ha, hence the fungicide@1500-1750 g/ha may be recommended for the control of downy mildew of grapes. The test fungicide was not phytotoxic to the grapevines.

**Keywords:** Azoxystrobin, bioefficacy, Downy mildew, grapes, Mancozeb, phytotoxicity

### INTRODUCTION

Grapevine (*Vitis vinifera* L.) is an important commercial fruit crop in sub-tropical regions of the world. India had a production of  $32 \times 10^5$  MT from an approximate average of  $1.5 \times 10^5$  ha in 2020-21 (Anonymous, 2021). Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Punjab are the major grapevine growing states of India (Dethle, 2000). Grapes are mainly consumed as fresh berries and also processed for making wine, juice, jelly/jam and raisins. Grape has many nutritional properties like high vitamins, minerals, lipids and fiber content along with antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic activities.

India exported  $2.6 \times 10^5$  MT of fresh grapes in 2021-2022 with a contribution of Rs.  $2.3 \times 10^5$  lakh in the national exchequer (Anonymous, 2021, 2022). Grapevine downy mildew caused by the fungal pathogen *Plasmopara viticola* Berk. & Curt. (Berl. and de Toni) is considered to have a high destructive potential causing 60-70% of crop loss.

(Sawant *et al.* 2010). Several fungicides like Propineb, Mancozeb, Fosetyl Al, Dimethomorph, Mandipropamid and Cyazofamid are reported to control the disease. (Ghule *et al.* 2018). The repeated use of solo fungicides with a single-site mode of action is associated with a higher risk of resistance evolution when compared to a more diversified approach, e.g., multiple fungicide classes in mixtures or in alternation (Bosch *et al.* 2014). Azoxystrobin 11% + Tebuconazole 18.3% SC gave a formidable control against the downy mildew of grapes (Reddy *et al.* 2019). The present investigation was carried out using a new formulation of a combination of Mancozeb 40% + Azoxystrobin 7% OS against the disease. Mancozeb [(FRAC code M 03) ethylene-bis-dithiocarbamate (EBDC)] is an extensively used fungicide that controls a wide variety of plant diseases and has not developed resistance to fungal diseases due to its multisite mode of action (Saha *et al.* 2022). For broad-spectrum disease management, mancozeb is often premixed with various systemic fungicides that have been reported to have developed resistance to *Plasmopara viticola*. Azoxystrobin [FRAC code 11, Quinone outside

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Inhibitor (QoI)] is a systemic strobilurin fungicide which inhibits cell respiration by binding to the protein subunits in the mitochondrial cytochrome bc1 complex (Fisher and Meunier, 2008). It was shown that rate of selection for resistance to azoxystrobin in *P. viticola* was delayed when the product was mixed with mancozeb (Gullino *et al* 2010). The efficacy of the combined action may be accentuated against the fungal pathogens with no risk of development of resistance against fungicides. Hence in the present study Mancozeb 40% + Azoxystrobin 7% OS was evaluated under field condition against downy mildew of grapes.

## MATERIALS AND METHODS

The field trial was conducted in a vineyard of Thompson Seedless located at Malgaon, Sangli (latitude: 16.86° N, longitude: 74.71° E, elevation: 584m MSL) for two consecutive seasons (2019-2020 & 2020-2021). The test chemical Mancozeb 40% + Azoxystrobin 7% OS branded as Prospell was supplied by Coromandel International Limited. Eight test treatments comprised of Mancozeb 40% + Azoxystrobin 7% OS @ 1250, 1500 & 1750 g/ha, Azoxystrobin 23% SC @ 500g/ha, Mancozeb 75% WP @ 2000g/ha, Dimethomorph 50 WP @ 500g/ha, Cymoxanil 8 % + Mancozeb 64% WP @ 2000g/ha and untreated control. The experiment was laid out in Randomized Block Design (RBD) with four replications in 8 grape vines with a spacing of 10 ft. x 6 ft. on Y trellises. Fungicide application was commenced with the visibility of initial symptoms (30 and 35 days after fruit pruning in 2018-19 and 2019-20 respectively) with knapsack sprayer. Total 4 sprays including one preventive spray were done. Water volume used for spray was calculated based on requirement of 1000 l/ha at full canopy. Downy mildew incidence on leaves was recorded visually adopting the 0-4 scale, where 0 = nil, 1 = trace to 25, 2 = 26 to 50, 3 = 51 to 75 and 4 = more than 75 leaf area infected (Horsfall and Heuberger 1942). Percent Disease Index (PDI) was calculated by the following formulae of McKinney (1923).

$$\text{PDI} = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of leaves observed} \times \text{Maximum of rating scale}}$$

The ratings on ten leaves were recorded on randomly selected canes. Ten such canes per vine were observed and 100 disease observations were recorded per replicate. Four replications for each

treatment were considered. Only actively growing downy mildew lesions were considered for recording ratings. The marketable yield from all the treatments was recorded at harvest and expressed in Kg /vine and further extrapolated to yield in t/ha basis.

The mean of PDI of both the seasons was calculated and percent disease control (PDC) was tabulated using the formula of Vincent (1947)  $I = C - T/C \times 100$ , where, I = percent disease control; C = PDI in untreated control; T = PDI in fungicide treatment

## Statistical Analysis

The PDI data was transformed by using arcsine transformation for leaves and analyzed statistically following Randomized Block Design (RBD) using Statistical Analysis System. All data obtained were subsequently analyzed statistically.

## Phytotoxicity

Phytotoxicity experiment was conducted at the same plot and the vines treated with sprays of different doses of Mancozeb 40% + Azoxystrobin 7% OS viz; 1500g and 3000g/ha. Vineyards were critically observed for presence of phytotoxic effects such as chlorosis, tip burning, necrosis, epinasty, vein clearing, hyponasty etc. on leaves and necrosis, russeting on berries after each spray of the fungicide. Observations were recorded at 0, 1, 3, 5, 7 and 10 days after spray of fungicides in the form of visual ratings in 0-10 scale where, 0 = No phytotoxicity, 1 = 0-10%, 2 = 11 – 20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, 10 = 91-100%.

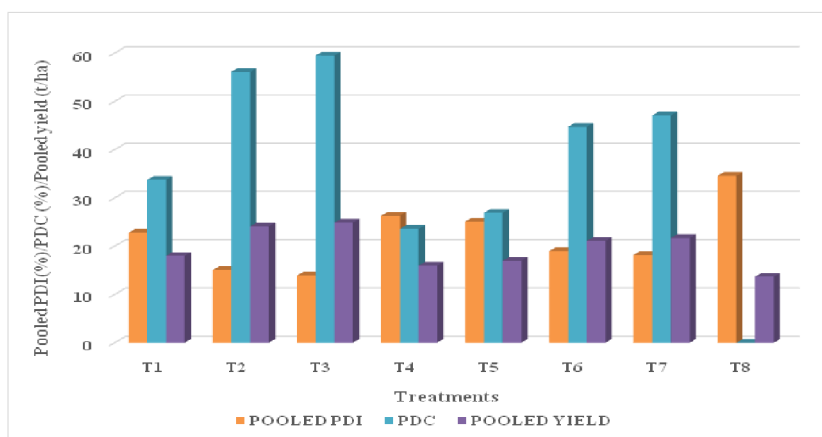
## RESULTS AND DISCUSSION

All treatments were significantly superior over untreated control for PDI on leaves and enhanced marketable yield/vine. The test fungicide Mancozeb 40% + Azoxystrobin 7% OS @ 1750g/ha, manifested the lowest PDI values i.e. 12.94 and 15.00 in 2019-20 and 2020–21 respectively. Similar trend was observed in pooled data with PDI 13.96 and PDC 59.50 (Table 1, Fig.1). It was followed by its lower dose i.e. 1500g/ha where the PDI values were 14.31 and 15.81 respectively in the two years of study with a mean PDC of

**Table1.** Bio-efficacy of Mancozeb 40% +Azoxystrobin 7% OS against downy mildew in grapes after fruit pruning

Treatments	Dose g/ha	PDI of downy mildew on leaves				Yield			
		2019-20	2020-21	Pooled	PDC	2019-20	2020-21	Pooled	
T1	Mancozeb 40% +Azoxystrobin 7% OS	1250	22.06 (28.00)	23.56 (29.03)	22.81 (28.52)c	33.75 (35.45)c	17.80	18.24	18d
T2	Mancozeb 40% +Azoxystrobin 7% OS	1500	14.31 (22.23)	15.81 (23.43)	15.06 (22.83)e	56.14 (48.53)a	23.81	24.43	24.1ab
T3	Mancozeb 40% +Azoxystrobin 7% OS	1750	12.94 (21.08)	15.00 (22.78)	13.96 (21.94)e	59.50 (50.47)a	24.73	25.02	24.9 a
T4	Azoxystrobin 23% SC	500	25.56 (30.37)	27.06 (31.34)	26.31 (30.85)b	23.58 (28.95)d	15.72	16.28	16 de
T5	Mancozeb 75%WP	2000	24.38 (29.58)	25.88 (30.57)	25.12 (30.08)b	26.92 (31.15)d	16.12	17.98	17 d
T6	Dimethomorph 50 WP	1000	18.25 (25.28)	19.75 (26.39)	19.12 (25.84)d	44.76 (41.98)b	20.08	22.08	21.1 c
T7	Cymoxanil 8 % + Mancozeb 64% WP	2000	17.44 (24.68)	18.94 (25.79)	18.18 (25.84)d	47.12 (43.34)b	20.71	22.63	21.7 bc
T8	Control	-	33.88 (35.56)	35.31 (36.45)	34.59 (36.01)a	0.00 (0.00)e	13.66	13.67	13.7e
CD (P = 0.05)		-	(1.34)	0.88	1.46		1.47	1.38	3.41

\*Figures in parentheses indicate arcsine transformed averages.



**Fig. 1:** Bio-efficacy of Mancozeb 40% +Azoxystrobin 7% OS against downy mildew in grapes after fruit pruning

56.14. Mancozeb 40% + Azoxystrobin 7% OS @1750g/ha and @ 1500g/ha were at par with each other. Component fungicides viz; Mancozeb 40% @2000g/ha and Azoxystrobin 7% OS@500g/ha exhibited a PDC of 26.92 and 23.58 respectively. This clearly shows the synergistic effect of Mancozeb 40% + Azoxystrobin 7% OS @1500g/ha and @1750g/ha. Standard check fungicide, Dimethomorph 50 WP@1000g/ha and Cymoxanil 8% + Mancozeb 64% WP@2000g/ha<sup>1</sup> exhibited a PDC of 44.76 and 47.12 respectively which were at par with each other. The untreated control had the maximum PDI of 33.88 and 35.31 in the two consecutive seasons under study. The reduction in disease by Mancozeb 40% + Azoxystrobin 7% OS was also reflected in the yield of the crop at the dose 1500 g/ha and 1750 g/ha which recorded 24.9 and 24.1 t/ha yield respectively. The untreated control exhibited 13.7 t/ha.

There was no occurrence of any phytotoxicity symptoms i.e. chlorosis, wilting, vein clearing, epinasty, hyponasty, necrosis and scorching on leaves up to 10 days after spray on any of the treatments considered in the study.

The test fungicide Mancozeb 40% + Azoxystrobin 7% OS @ 1750g/ha, manifested the lowest PDI values in both 2019-20 and 2020-21 i.e. 12.94 and 15.00 respectively with a mean percent disease control (PDC) of 59.50. Wong and Wilcox (2001) reported that azoxystrobin was the only available fungicide to provide effective control of downy mildew (*Plasmopara viticola*) in addition to powdery mildew (*Uncinula necator*). Wicks and Hitch (2002) evaluated the strobilurin fungicide, Amistar (azoxystrobin) for control of downy mildew of grapes and recorded that Amistar @ 0.5g/l was more effective than all treatments. Hundekaret *et al.* (2008) reported that Kresoxim methyl which is in the same group of azoxystrobin was also found to be effective in reducing the severities of both powdery and downy mildew. However, repeated use of azoxystrobin resulted in fungicide resistance (Sawant *et al.* 2015) and there was a need for a proper ready-mix fungicide to be designed and mancozeb was an exact fit for this purpose. Azoxystrobin 23% SC was found effective for the control of late blight of potato caused by *Phytophthora infestans* (Mandal *et al.* 2018) and downy mildew cucumber caused by *Pseudoperonospora cubensis* which were oomycetous pathogen like *Plasmopara viticola*

(Anand *et al.* 2008). Mancozeb is known to control downy mildew and anthracnose diseases of grapes (Saha *et al.* 2022). Although the non-systemic fungicides viz., Mancozeb, Captan and Ziram exhibited statistically inferior efficacy as compared to systemic ones (Khilari *et al.* 2010), they were the best for ready mix fungicides to mitigate resistance issues. Six applications (one per week) of azoxystrobin (AZ) solo or in mixture with mancozeb provided excellent control of downy mildew. In the present study also, the ready mix fungicide formulation not only gave a good control against downy mildew, but also exhibited synergistic effect in the management of the disease. Similar synergistic effect was reported by Saha *et al.* (2014) with another combination of a QoI fungicide viz; Trifloxystrobin with tebuconazole for the management of early blight of tomato. Sendhilvel *et al.* (2004) also found that there was no leaf injury on grapevine at a higher concentration of azoxystrobin, which is aligned to the present investigation.

## CONCLUSION

Mancozeb 40% + Azoxystrobin 7% OS @ 1500-1750 g/ha as a foliar spray manifested significantly higher disease control of downy mildew in grapes over its solo doses, increased the yield and were devoid of any phytotoxic effects on grapes. Thus, these combinations at above doses may be recommended for the management of downy mildew of grapes.

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