AATCC Review

20 June 2025: Received
08 August 2025: Revised
16 August 2025: Accepted
15 September 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Synergistic Effect of Amisulbrom 5.63% + Zoxamide 11.25% SC in Downy Mildew Control in Grapes

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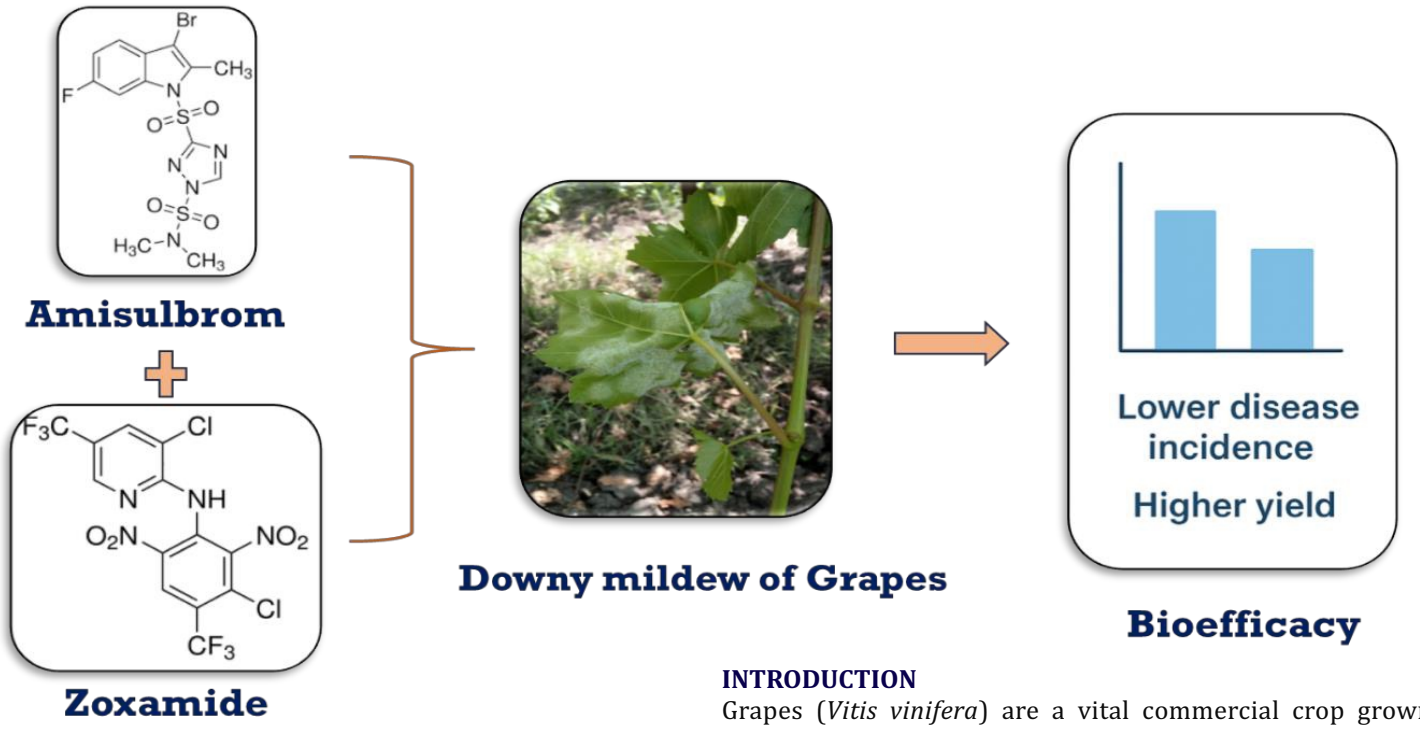
ABSTRACT

Downy mildew (c.o. Plasmopara viticola) is an important fungal disease affecting grapevines, leading to severe yield losses globally. The increasing dependence on fungicides for managing the disease has raised concerns regarding the development of resistance in pathogen populations. Hence, there is an incessant need for new fungicides or their combination to manage downy mildew effectively. Fungicides utilized in the control of P. viticola exhibit a range of modes of action, which can be both broad-spectrum as well a specific to the pathogen. The efficacy of novel fungicide Amisulbrom 5.63% + Zoxamide 11.25% SC against downy mildew of grapes was evaluated for two seasons at Nashik district, Maharashtra, India and it was found that the fungicide @ 1000 ml/ha was effective in reducing the disease. Further the phytotoxicity was not observed in the test chemical- treated plots of grapevine @ 1000 and 1250 ml/ha. The yield of grape berries was also high in the aforementioned dose as compared to the untreated control. The study faced challenges due to variable environmental conditions that promoted rapid downy mildew spread and made it difficult to maintain uniform disease pressure. Nevertheless, it establishes the field efficacy and safety of Amisulbrom + Zoxamide against P. viticola, while also confirming its yield-enhancing potential without phytotoxic effects.

Keywords: *Bio efficacy, Grapes, Amisulbrom, Zoxamide, Downy mildew, Phytotoxicity, Per cent disease Index, Per cent disease Control.*

Graphical abstract



INTRODUCTION

Grapes (*Vitis vinifera*) are a vital commercial crop grown worldwide, significantly enhancing agricultural productivity and providing economic benefits to farmers [1]. In 2022–2023, global grape production amounted to 27.9 million metric tonnes, while India produced 3780.81 thousand metric tonnes across 171.32 thousand hectares in 2023–24 [2].

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.04.420

However, Indian viticulture is affected by significant challenges from various biotic and abiotic stresses, among which downy mildew, caused by the oomycete pathogen *Plasmopara viticola*, is one of the most destructive diseases [3].

This pathogen can lead to substantial yield losses and reduced grape quality, particularly in regions with humid and warm climates that favor its proliferation. Downy mildew primarily affects the leaves, stems and fruits of grapevines, manifesting as yellowish oil spots on the upper surface of leaves, which later develop into white, downy fungal growth on the underside. (fig. 1) [4]. Severe infections can cause premature defoliation, reduced photosynthesis and significant crop losses. Effective management of downy mildew is therefore essential for sustainable grape production. Over the years, fungicidal applications remained the nucleus for controlling downy mildew in vineyards. Several fungicides viz., Mancozeb 40% + Azoxystrobin 7% OS [5], Oxathiapiprolin 48 g + Amisulbrom 240 g/ L SE [6], Oxathiapiprolin 0.6% + Mancozeb 60% [7], oxathiapiprolin (3%) + Mandipropamid (25%) w/v (280 SC) [8], Amilsulbrom 20% SC [9] were effective for controlling downy mildew disease of grapes. However, fungicide resistance due to the G143A point mutation in the cytochrome b gene of *Plasmopara viticola* isolates has become a global problem [10, 11]. The continuous search for new molecules brought to the fore amisulbrom + zoxamide which could be used to manage the diseases.

Fig. 1: Symptoms of downy mildew of leaves

Amisulbrom belongs to the QII (quinone inside inhibitor) group [12] targeting the mitochondrial complex III, thereby disrupting energy production in oomycetes, and it was found effective against downy mildew of grapes. [9]. Zoxamide, on the other hand, inhibits β-tubulin assembly in the cytoskeleton, interfering with cell division in oomycete pathogens. [13]. The combination of these two active ingredients could provide a synergistic effect, enhancing efficacy against downy mildew and delaying the onset of fungicide resistance. The integration of amisulbrom and zoxamide could provide a holistic solution, given their complementary mechanisms and low environmental persistence. However, comprehensive field studies are required to evaluate their combined efficacy under diverse agro-climatic conditions, as well as their phytotoxicity effects and influence on the grape.

This study evaluates the bio-efficacy of amisulbrom + zoxamide formulations against downy mildew in grapes, aiming to determine optimal application rates and schedules for effective sustainable disease management.

Material and Methods

Experimental Set up: The experimental trials were conducted during the fruiting seasons (October-February) of 2020–21 and 2021–22 at Warkheda, Nashik, Maharashtra, (latitude 20.53° N and a longitude of 75.16° E, 565 m above MSL). The test chemical Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v (supplied by Dhanuka Agritech Limited). Amisulbrom 20% SC @375 ml/ha, Metiram 44%+Dimethomorph 9% WG @2500 g/ha, and Iprovalicarb 5.5% + Propineb 61.25% WP @ 1000 g/ha were the standard check fungicides used in the study. A water spray control was maintained as well.

The study was performed in a 10-year-old vineyard of the Thompson Seedless cultivar, planted at a spacing of 6 ft × 10 ft and trained on a "Y" trellis system oriented in the north-south direction. The experiments followed a randomized block design (RBD) comprising eight treatments with four replications each. The vines selected for the experiment were subjected to natural infection of downy mildew. Four sprays, including one preventive spray, were given whenever the weather conditions were favorable for disease development. The first spray was carried out when the disease infection was observed in the untreated control plot. The water volume used for spray was calculated based on the requirement of 1000 L/ha at the full canopy. A knapsack sprayer with a hollow cone nozzle was used for spraying.

Foliar Disease Intensity: The incidence of downy mildew was recorded on leaves, and the intensity of downy mildew on plant leaves was recorded by 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. [14] PDI was calculated using the following formula by [15]:

$$\text{Per cent Disease Index} = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of leaves observed} \times \text{Maximum of rating scale}}$$

The per cent disease control (PDC) was calculated by using the formula,

$$\text{PDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

The ratings on ten leaves were recorded on randomly selected canes. Ten such canes per vine were observed; thus 100 disease observations were recorded per replicate. Four replications for each treatment were considered. Only actively growing downy lesions were considered for recording the ratings.

Phytotoxicity

A Phytotoxicity experiment was conducted at the same plot, and the vines were treated with sprays of Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v @1000 ml/ha and 1250 ml/ha. Vineyards were critically observed for the presence of phytotoxic effects such as chlorosis, tip burning, necrosis, epinasty, vein clearing and hyponasty 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%.

Marketable Yield

To calculate the total marketable yield, fruits were harvested from each treatment in four replications, including the untreated control plants and the yield was calculated in Q/ha.

Statistical Analysis

The PDI data were transformed using an arcsine transformation for leaves and bunches, and it was analyzed statistically following Randomized Block Design (RBD) using a Statistical Analysis System WASP 2.0 (Central Coastal Agricultural Research Institute). The yield data were analyzed without transformation. Means were compared using the Least Significant Difference (LSD) Test.

RESULT

Table 2. Bio efficacy of Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v against downy mildew of grapes during 2020-21 and 2021-22

Tr. No.	Treatment	Dosage		PDI of downy mildew on leaves		Pooled PDI	Pooled PDC	Yield (t/ha)		Pooled Yield (t/ha)
		g a.i.	Formulation	2020-21	2021-22			2020-21	2021-22	
T1	Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v	42.25+84.37	750	16.75 (24.13) c	17.75 (24.90) c	17.25 (24.52) c	55.14 (47.95) c	15.55 d	17.75 c	16.65 d
T2	Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v	56.30 + 112.50	1000	11.13 (19.43) a	12.50 (20.66) a	11.81 (20.06) a	69.42 (56.46) a	24.48 b	26.44 a	25.46 a
T3	Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v	70.37 + 140.62	1250	10.63 (18.97) a	11.94 (20.17) a	11.28 (19.59) a	70.80 (57.32) a	26.81 a	28.65 a	27.73 a
T4	Amisulbrom 20% SC	75	375	13.88 (21.85) b	14.88 (22.68) b	14.38 (22.28) b	62.59 (52.29) b	20.08 c	22.15 b	21.12 c
T5	Zoxamide 24% SC	150	625	14.31 (22.21) b	15.51 (23.18) b	14.91 (22.71) b	61.13 (51.44) b	19.78 c	21.62 b	20.70 c
T6	Metiram 44%+Dimethomorph 9% WG	1100 + 225	2500	18.19 (25.23) c	19.88 (26.47) c	19.03 (25.87) d	50.46 (45.26) d	12.69 e	14.57 d	13.63 e
T7	Iprovalicarb 5.5% + Propineb 61.25% WP	55 + 612.50	1000	20.25 (26.73) d	20.63 (27.00) d	20.44d (26.88) d	46.63 (43.07) e	10.86 f	12.98 de	11.92 e
T8	Untreated control	-	-	35.31 (36.43) e	41.94 (40.35) e	38.63 (38.42) e	0.00 (2.89) f	7.51 g	10.70 e	9.10 f
CD (*P = 0.05*)				1.19	1.11	1.14	2.16	1.66	2.67	1.89
SE ± (m)				0.40	0.38	0.38	0.78	0.56	0.91	0.64

Figures in parenthesis are arc-sin transformed values.
Figures in each column followed by same alphabet (s) are not significantly different

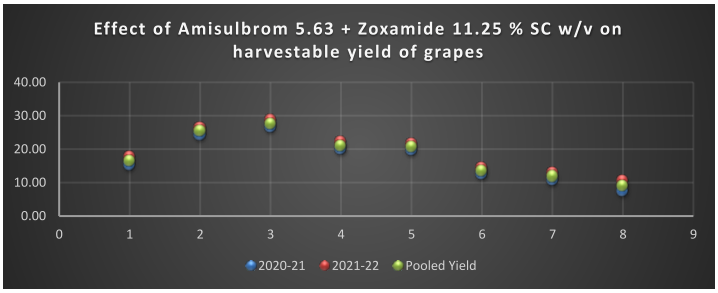

Fig. 2: Effect of Amisulbrom 5.63 + Zoxamide 11.25 % SC w/v on harvestable yield of grapes

The pooled percent disease index (PDI) of downy mildew on leaves varied significantly among the treatments. The lowest Pooled PDI (11.28) was recorded in T3, followed by T2 (11.81), indicating the highest efficacy in disease suppression. Both treatments showed significantly better disease control as compared to other fungicidal treatments and the untreated control (T8), which exhibited the highest PDI (38.63).

The pooled percent disease control (PDC) was also highest in T3 (70.80%), followed closely by T2 (69.42%), demonstrating the superior effectiveness of these treatments in managing downy mildew. In contrast, the untreated control (T8) recorded the lowest disease control (2.89%). Among the stand-alone fungicidal treatments, T4 and T5 provided moderate disease control, with pooled PDIs of 14.38 and 14.91, with corresponding PDCs of 62.59 and 61.13, respectively. However, their effectiveness was significantly lower than the combination treatments (T2 and T3).

The different treatments had a substantial effect on grape yield. The highest pooled yield (27.73 t/ha) was obtained in T3, followed by T2 (25.46 t/ha), both of which were statistically superior to all other treatments.

The increased yield in these treatments can be attributed to the effective control of downy mildew, reducing disease-induced losses and maintaining vine health. Stand-alone fungicide applications, T4 (21.12 t/ha) and T5 (20.7 t/ha), resulted in moderate yields, further emphasizing the advantage of combination fungicides over single-active ingredient formulations. The lowest yields were recorded in T7 (11.92 t/ha) and T6 (13.63 t/ha), indicating relatively lower efficacy in disease management. The untreated control (T8) had the lowest yield (9.10 t/ha), highlighting the significant yield losses associated with severe downy mildew infection.

DISCUSSION

Fungicides play a crucial role in managing downy mildew;, but the overuse of fungicides has led to resistance, challenging their effectiveness in managing downy mildew. Thus, investing in research for new fungicide formulations is crucial for sustainable disease control [16]. The results of this study demonstrated that the combination of Amisulbrom 5.63% + Zoxamide 11.25% SC at 1050 ml/ha manifested the most effective protection against downy mildew (*Plasmopara viticola*) in grapevines, resulting in the highest grape yield among all treatments. This outcome is consistent with the findings of Bacci *et al*. [17], who reported that the combination of Zoxamide with other fungicides, such as Mancozeb, provided superior control over downy mildew. Saha *et al*. [18] reported that foliar application of Zoxamide + Cymoxanil at 500–600 g/ha and Zoxamide + Mancozeb at 2250–2500 g/ha resulted in significantly improved control of early and late blight diseases in tomato. The combination treatment in the present study not only minimized disease incidence but also optimized yield, further emphasizing the effectiveness of this dual-action approach for disease control.

Amisulbrom, as a translaminar and curative fungicide has demonstrated its potential in managing downy mildew in grapes.

Sawant *et al*. [9] showed that Amisulbrom 20% SC, at a dosage of 375 ml/ha, significantly reduced disease incidence and enhanced yield by effectively controlling downy mildew. These findings are corroborated by the present results, where the combination of Amisulbrom and Zoxamide achieved effective disease control. Moreover, Honda *et al*. [19] demonstrated the strong efficacy of Amisulbrom 200g/L SC in controlling potato late blight in Europe and Japan. The fungicide exhibited long-lasting prevention, rapid leaf penetration and excellent rain fastness. Zoxamide has shown strong activity in controlling a wide range of oomycete diseases [20, 21]. Amisulbrom has been reported to reduce downy mildew infection by inhibiting the zoospores of the pathogen and limiting sporangial growth. When combined with zoxamide, the two fungicides exhibit a synergistic effect, enhancing their overall efficacy. Present investigations supported these findings, showing that while standalone applications of Amisulbrom and Zoxamide provided moderate control, their combination significantly enhanced disease management. The increased efficacy of the combination treatment suggested that the dual-mode of action from both fungicides provides a more comprehensive defence against the pathogen, compared to when either fungicide is used alone. Similar results were shown by Pharate *et al*., [6] that a combination of Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml /ha and 375.00 ml/ha controlled downy mildew of grapes. Due to their unique modes of action and the co-formulation with a multisite fungicide, this combination presented a promising new alternative for managing downy mildew of grapes. Hence, the combination of Amisulbrom 5.63% + Zoxamide 11.25% SC is a novel option against downy mildew of grapes.

CONCLUSION

The combination of Amisulbrom 5.63% + Zoxamide 11.25% SC @1000 ml/ha proved to be highly effective in controlling downy mildew and enhancing grape yields. The use of multi-mode action fungicides in disease management not only improved productivity but also contributed to the sustainability of grape cultivation. This study provided practical recommendations for grape growers, emphasizing the importance of strategic fungicide applications in mitigating the economic impact of downy mildew and promoting sustainable viticulture practices.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR NRCG, for her keen interest and enthusiastic participation in the study. They are also grateful to Dhanuka Agritech Limited for providing the samples of Amisulbrom 5.63% + Zoxamide 11.25% SC.

DECLARATIONS

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors declare no potential conflicts of interest

Author Contributions

This work was carried out in collaboration among all authors. Authors SS and SP planned the research work. Author SK carried out a field study. Authors SP and SS prepared the manuscript and actively participated in the discussion and revision of the manuscript. SP and SS read and approved the final manuscript.

Competing interests

We don't have any competing interests. All authors have agreed to publish.

Future Scope of the study

The current results demonstrate that Amisulbrom 5.63% + Zoxamide 11.25% SC is effective against grape downy mildew; nevertheless, more research is required to support its use in viticulture. Its constancy under various agro-climatic circumstances would be confirmed by multi-location and multi-season trials involving a variety of grape cultivars. Long-term monitoring of *P. viticola* populations is essential to assess resistance development and ensure sustainable use.

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