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Study of New Combination Fungicide Oxathiapiprolin 48 g + Amisulbrom 240 g/ L SE against Downy Mildew of Grapes in India

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Authors' 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 trial. Authors SP and SS jointly prepared the manuscript and actively participated in the discussion and revision of the manuscript. Author BSG provided a sample. All authors read and approved the final manuscript.

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ABSTRACT

Grapes suffer from various diseases and amongst them, downy mildew (c.o. *Plasmopara viticola*) causes global crop losses to the extent of 80-90%. Several fungicides are used to control these diseases, but the major risks involved in using fungicides are development of fungicide resistance as well as environmental pollution issues. This necessitates the evolution of new molecules with a unique mode of action. Oxathiapiprolin and amisulbrom are novel options with a unique mode of

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actions. A field study was conducted to evaluate the efficacy of combination fungicide Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE against downy mildew during 2020-21 and 2021-22 at Sangli, Maharashtra. Total four foliar sprays (one preventive + three curative) were applied as soon as the disease appeared in experimental plots.

Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml /ha and 375.00 ml /ha were at par with each other and recorded significantly lowest pooled percent Disease Index i.e. 19.11 and 19.25 respectively with corresponding percent disease control of 66.72 and 66.35 over untreated control (PDI of 36.15). In case of harvestable yield Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml /ha and 375.00 ml/ha showed the highest marketable yield of 26.49 and 25.80 t/ha respectively.

Keywords: Bio efficacy; fungicides; downy mildew; oxathiapiprolin; amisulbrom.

1. INTRODUCTION

Grape (*Vitis vinifera*) is an important commercial fruit crop of India and is responsible for maximum foreign exchange among all fruit crops. India recorded a grape production of 3490 thousand MT from an average area of 162 Thousand ha in the year 2021-22 [1]. “Along with economic value, grapes are also endowed with several nutritional attributes. Grape is a rich source of bioactive molecules including phenolic acids, flavonoids, anthocyanins, stilbenes and lipids. Reports suggested that grapes had various antioxidant, antimicrobial, anti-inflammatory and anti-carcinogenic activities with a broad spectrum application in food and nutraceutical industries” [2]. “However, the productivity is challenged by severe biotic factors and diseases hold the stage centre among them. Downy mildew caused by the obligate biotrophic oomycete *Plasmopara viticola* is responsible for global crop losses to the maximum extent and it is one of the most serious disease of grapevines worldwide” [3]. “*Plasmopara viticola* produces asexual, biflagellate zoospores and sexual oospores which is the resting stage of the pathogen. Oospores represented the primary inoculums and sporangia served as a secondary spread of the pathogen. Zoospores form within the sporangia which are disseminated by wind and rain splash. Zoospores released from the sporangia swim in free water on the grapevine surface and start the infection” [4].

“The disease developed intensively in humid climatic conditions i.e. 18-25^{0}C temperature and a relative humidity 80-90%. The first symptom of disease appeared on the new flush as small light green patches on the upper surface of the leaves and a whitish downy growth on the corresponding lower surface. The downy growth of fungus spread rapidly on the lower surface which turned corresponding greenish patches on the upper surface yellow and chlorotic (oil spots) with age” [5].

“The disease leads to sugar content reduction, causes failure of many physiological processes in plants, leaf drop and suppression of vines, and poor ripening of shoots, which substantially affects yield. Yield losses due to downy mildew reach 70% in certain years in case of epiphytotic development of the disease” [6,7,8]. For control of downy mildew diseases many chemicals such as Propineb, Mancozeb, Fosetyl Al, Dimethomorph, Mandipropamid and Cyazofamid [9] and Amisulbrom [10] were used. “Grapevine accounts for the largest proportion of fungicides used in the European Union and the main causes of large-scale fungicide use in viticulture are powdery mildew and downy mildew” [11]. Chemical control remains the method of choice in terms of grapevine protection against downy mildew, as the most effective and less laborious approach.

“Oxathiapiprolin (FRAC code 49) affects the oomycetous fungi by inhibiting an oxysterol binding protein (OSBP) homologue. Oxathiapiprolin is a systemic fungicide that, when applied to a plant, binds to the waxy coating of the leaves and maintains the required concentration inside the plant, by penetrating the leaf tissues adjacent to the cuticle, and systemically spreads through the plant, entering the xylem vessels. The mechanism of its action consists in affecting the Oxysterol-binding protein at the molecular level; blocking of this protein first leads to arrested growth of the fungal mycelium and germ tubes of spores and then to the death of the fungus” [12].

Amisulbrom (FRAC code 21) targets the same group of pathogens by inhibiting mitochondrial respiration and it has protective properties by working in the waxy cuticle of the plant [13]. In the present study, the combination of fungicide

Oxathiapiprolin + Amisulbrom was evaluated for its bio efficacy against downy mildew of grapes under field conditions.

2. MATERIALS AND METHODS

The bioefficacy of combined fungicide viz. Oxathiapiprolin 48 g + Amisulbrom 240 g/ L SE @ 250 ml/ha, 312.5 ml/ha and 375 ml/ha were evaluated against downy mildew infection on grape leaves along with its solo components Oxathiapiprolin 10 OD @ 400 ml/ha and Amisulbrom 20% SC @ 375 ml/ha. The field trial was conducted in a vineyard of Thompson Seedless located at Malgaon, Sangli for two consecutive seasons 2020-21 and 2021-22 after fruit pruning. The test chemical Oxathiapiprolin 48 g + Amisulbrom 240 g/ L SE and Oxathiapiprolin 10.1% w/w OD was supplied by Syngenta India Pvt. Ltd. Amisulbrom 240 g/ L SE and Mandipropamid 23.4% SC were the standard check fungicides and water sprayed untreated control was maintained as well. 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 started with the visibility of initial symptoms (30 and 35 days after fruit pruning in 2020-21 and 2021-22 respectively) with knapsack sprayer. Total 4 sprays including one preventive spray were given at an interval of 10 days to vines. 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 [14]. Percent Disease Index (PDI) was calculated by the following formulae of McKinney [15].

PDI = (Sum of numerical ratings × 100)/(Number of leaves observed × 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/ha basis.

The mean of PDI of both the seasons was calculated and percent disease control was tabulated using the formula of Vincent [16].

$$I = C\text{-}T/C \times 100$$

Where,
I=percent disease control; C=PDI in untreated control; T= PDI in fungicide treatment

2.1 Statistical Analysis

The PDI data was transformed by using arcsine transformation for leaves and analyzed statistically following Randomized Block Design (RBD) using WASP 2.0 (Central coastal Agricultural Research Institute). All data obtained were subsequently analyzed statistically.

3. RESULTS

The two doses of Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml/ha and 375 ml/ha gave a significant control of downy mildew of grapes with a significant increase in yield over its solo doses as well as the untreated control (Table 1). In 2020-21, the test fungicide Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 375 ml/ha, manifested the lowest PDI of 20.04 followed by Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml/ha which a recorded PDI of 20.15. Solo fungicides viz; Oxathiapiprolin 10.1% w/w OD @ 400 ml/ha and Amisulbrom 20% SC @ 375 ml/ha recorded a PDI of 22.78 and 21.45 respectively. Standard check fungicide, Mandipropamid 23.4% SC @ 800 ml/ha showed a PDI of 24.39.

In 2021-22, result showed that all the treatments were observed to be significantly superior over untreated control in minimizing PDI of downy mildew on leaves. The test fungicide Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 and 375 ml/ha recorded a PDI of 18.31 and 18.13 respectively. Solo fungicides Oxathiapiprolin 10.1% w/w OD @ 400 ml/ha, Amisulbrom 20% SC @ 375 ml/ha, and Mandipropamid 23.4% SC @ 800 ml/ha showed PDI 21.13, 19.64 and 22.78 respectively. The untreated control had the maximum PDI of 36.45 and 35.85 in the two consecutive seasons respectively under study. A similar trend was deduced in pooled data where in the test fungicide Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 and 375 ml/ha recorded a pooled PDI of 19.25 and 19.11 respectively.

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Table 1. Bio efficacy of oxathiapiprolin 48g + amisulbrom 240 g/ L SE against downy mildew disease and yield of grapes in India

Tr. No	Treatments	Dose/ha Formulation (ml)	2020-21	2021-22 Pooled Data		Percent Reduction over control	Yield (t/ha)			
							2020-21	2021-22	Pooled Data	Percent Increase over control
T_1	Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE	250	18.81 (25.70)e	16.81 (24.20)e	17.81 (24.96)e	48.64 (44.21) e	15.13 e	16.61 c	15.87 d	19.45 (24.48)
T_2	Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE	312.5	11.88 (20.15)a	9.88 (18.31)a	10.88 (19.25)a	68.67 (55.97) a	24.95 a	26.66 a	25.80 a	50.22 (45.13)
T_3	Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE	375	11.75 (20.04)a	9.69 (18.13)a	10.72 (19.11)a	69.15 (56.26) a	25.38 a	27.59 a	26.49 a	51.92 (46.11)
T_4	Mandipropamid 23.4% SC	800	17.06 (24.39)d	15.00 (22.78)d	16.03 (23.60)d	53.88 (47.22) d	16.77 d	18.08 c	17.43 cd	25.83 (29.47)
T_5	Oxathiapiprolin 10.1% w/w OD	400	15.00 (22.78)c	13.00 (21.13)c	14.00 (21.97)c	59.72 (50.61)c	18.39 c	18.99 c	18.69 c	31.64 (33.95)
T_6	Amisulbrom 20 % SC	375	13.38 (21.45)b	11.31 (19.64)b	12.34 (20.56)b	63.96 (53.44) b	21.21 b	21.75 b	21.48 b	40.59 (39.50)
T_7	Untreated Control	-	35.31 (36.45)f	34.31 (35.85)f	34.81 (36.15)f	-	13.62 e	11.91 d	12.76 e	-
CD (*P* = 0.05)		**-**	**0.89**	**1.07**	**0.98**	**1.89**	**1.16**	**2.61**	**1.95**	**6.18**

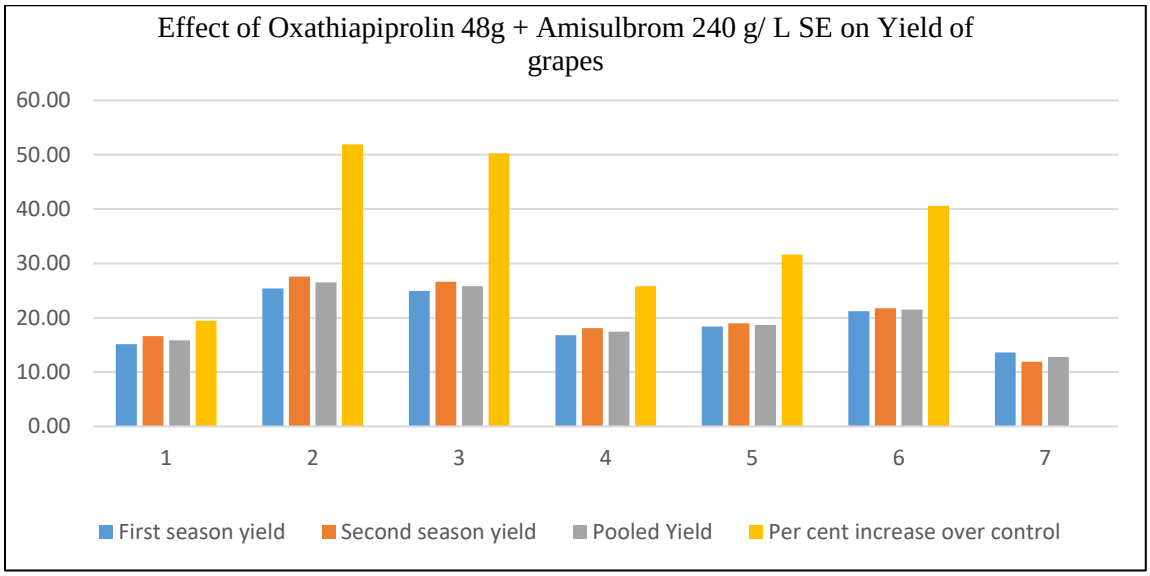

Fig. 1. Percent disease index and percent disease control of downy mildew by oxathiapiprolin 48g + amisulbrom 240 g/ L SE

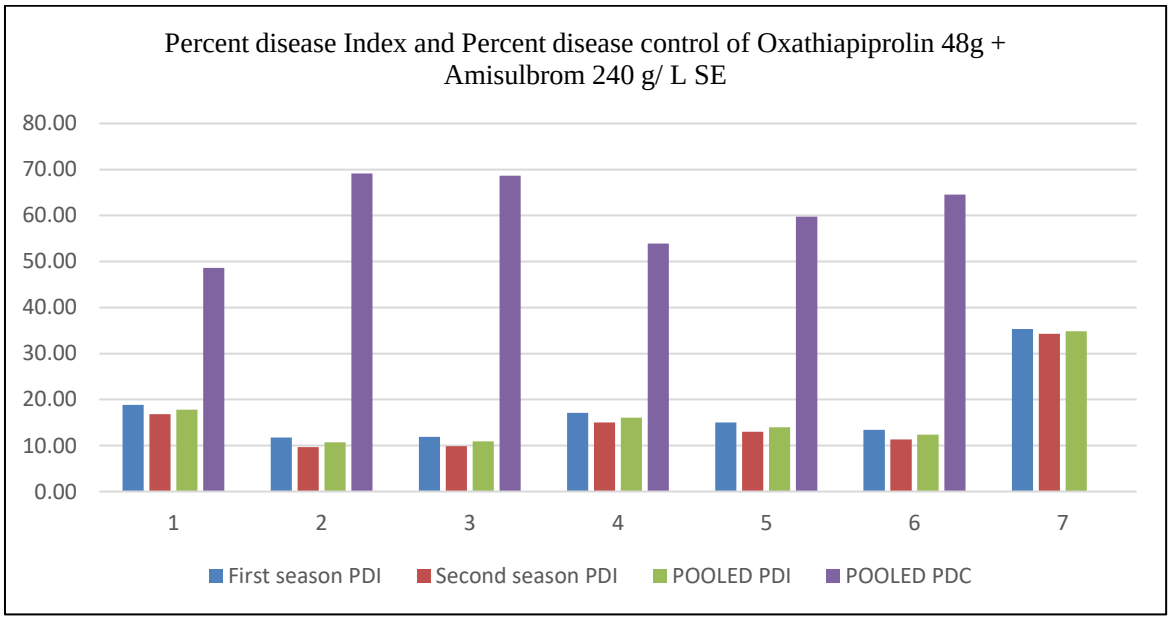

Fig. 2. Effect of oxathiapiprolin 48g + amisulbrom 240 g/ L SE on yield of grapes

The harvestable yield of grapes in 2020-21, was highest in case of Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 and 375 ml/ha which recorded the highest marketable yield i.e. 24.95 t/ha and 25.38 t/ha respectively. It was followed by Amisulbrom 20% SC, Oxathiapiprolin 10.1% w/w OD and Mandipropamid 23.4% SC which recorded a yield of 21.48 t/ha, 18.69 t/ha and 17.43 t/ha respectively. The same trend was observed in the second season wherein Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 and 375 ml/ha had a marketable yield higher than all other treatments.

4. DISCUSSION

In the present study, three Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE concentrations and its solo components were evaluated for their field efficacy in the control of downy mildew of grapes. According to [12,17] solo performance of Oxathiapiprolin was used to control the major

oomycetes diseases of grapes, potatoes, and vegetables. Oxathiapiprolin showed better preventive and curative management of potato late blight. [18,19] reported that Oxathiapiprolin was effectively inhibiting all the developmental stages in the asexual life cycle of *Pseudoperonospora cubensis* the downy mildew causal agent in cucurbit leaves [18] suggested that "oxathiapiprolin mixtures (with azoxystrobin, Mandipropamid or mefenoxam) were reported to be highly effective against *Phytophthora infestans* in tomato and *Pseudoperonospora cubensis* in cucumber".

Envisaging the problem of fungicide resistance it was necessary to evaluate the bio efficacy of a new fungicide amisulbrom against downy mildew. Amisulbrom is a QiI (Quinone inside Inhibitor) fungicide. Sawant et al. [10] reported that "Amisulbrom 20% SC @ 375 ml/ha may be considered as an effective dose to control the downy mildew of grapevine on leaves and bunches and increase yield as well. Key characteristic of amisulbrom is that it quickly penetrated into wax layers of plant leaf, hence was not affected by rainfall. In addition to that amisulbrom had a significant effect on the viability of zoosporangia at 4 days curative application, which indicated the inhibition of secondary infection".

The results showed that the combination fungicide Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 375 ml/ha manifested lower PDI (19.25) and higher PDC (66.35) which was significantly superior over rest of the treatments. It showed that the mixture of two fungicides is more effective than the solo fungicide and untreated control, which is in alignment with the study performed by the Gisi and Cohen [20] who showed that different modes of action were crucial for the performance and interaction of two fungicides in a mixture. The mixtures always performed better than solo oxathiapiprolin, chlorothalonil, azoxystrobin and mandipropamid. Same results were reported by Rashid et al. [21] where in superiority of combination formulations (Dimethomorph 9% + Mancozeb 60%, Phenomadone + Mancozeb (0.2%) and Mancozeb 63% + Carbendazim 12%) over the solo application (Mancozeb, Carbendazim and Propiconazole) in Chickpea.

The combination of oxathiapiprolin 48g+Amisulbrom 240 g/L SE @ 312.5 ml/ha gave an excellent control against downy mildew of grapes. In similar lines Oxathiapiprolin + Amisulbrom was ready mixed not only to negate resistance issues but also to have a sustainable, durable control of downy mildew.

5. CONCLUSION

Oxathiapiprolin 48g + Amisulbrom 240 g/ L SE @ 312.5 ml/ha as a foliar spray manifested significantly higher disease control of downy mildew in grapes, increased the yield and were devoid of any phytotoxic effects on grapes. Therefore, using these combinations at the above dosages may be advised for the treatment of grape downy mildew.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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