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SENSITIVITY ASSESSMENT OF *PLASMOPARA VITICOLA* AGAINST MEFENOXAM UNDER IN VITRO CONDITIONS

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ABSTRACT

Plasmopara viticola, the causal agent of downy mildew, is one of the most dreadful pathogens of grapes in the global paradigm. Phenylamide fungicides are a highly active class of fungicides that target oomycetous pathogens, and Plasmopara is not an exception. The present study was undertaken to assess the sensitivity of field isolates of *P. viticola to mefenoxam*. From July 2020 to August 2021, five hundred downy mildew-affected samples were collected from the Nashik, Sangli, Solapur, and Pune districts of Maharashtra as well as Bijapur and Bengaluru from Karnataka. The experiment was conducted at the ICAR-National Research Centre for Grapes Laboratory via the 24-well plate leaf disc assay method. The concentrations used in the study were 0, 0.01, 0.1, 1, 3, 10, and 100 ppm. The highest concentration at which the isolates sporulated was noted. Only 8 samples from Nashik, 2 samples from Sangli, 1 sample from Solapur, 7 samples from Pune, 4 samples from Bijapur, and 5 samples from Bengaluru presented symptoms at 1 ppm. No symptoms were observed at 3, 10, or 100 ppm in any of the isolates. Notably, 5.4% of the isolates were resistant to mefenoxam at 1 ppm. The resistance of Plasmopara viticola to Mefenoxam in all the grape-growing regions suggested the need for a premix of this fungicide with a contact fungicide for more effective control of downy mildew in grapes.
***Keywords*:** grapevine; downy mildew; oomycete; fungicide resistance; Mefenoxam.

Introduction

Grapes are an exportable commodity in India and constitute a significant share of the national exchequer. The estimated area for the production of grapes in India from 2022--2023 was approximately 162 thousand hectares (Anonymous, 2023). The downy mildew caused by *Plasmopara viticola* is regarded as the most detrimental grapevine disease because of the enormous production losses caused by this disease and the expense of preventive treatments. (Buonassisi *et al*., 2017). Under favorable weather conditions, the pathogen can infect all the leaves and bunches of a vine if it is not adequately controlled (Toffolatti *et al*., 2018). Several fungicides, such as propineb, mancozeb, fosetyl Al, dimethomorph, mandipropamid and cyazofamid, have been reported to control this disease (Ghule *et al*., 2018). Multiple groups of fungicides, classified as high, moderate- or low-risk fungicides for the development of resistance in *P. viticola*, constitute the major arsenal to combat the pathogen. In India, single target site fungicides of quinine outside inhibitors (QoIs); carboxylic acid amides (CAAs); phenyl amides (PAs) (benalaxyl, metalaxyl-M); cyanoacetamide-oximes (cymoxanil) groups; and multitarget site fungicides, such as chlorothalonyl, mancozeb, phosphonates, and copper-based fungicides, are registered for downy mildew management in grapes (Anonymous, 2018-19; APEDA, 2009).

Phenylamide fungicides (FRAC group 4) are a highly active class of fungicides that target oomycetous pathogens, including *Plasmopara viticola*. Phenylamides (PAs) prevent oomycete pathogens from producing ribosomal RNA (rRNA), which prevents the development of sporangia, hyphal growth, and other oomycete life stages. The development of fungicide

resistance is more likely to occur with phenylamide fungicides, as research has demonstrated that PA resistance is controlled by a single incompletely dominant gene, while multiple mutations or mechanisms may be involved in the development of PA resistance. (Hermann *et al.,* 2019).

Mefenoxam is a systemic fungicide belonging to the phenylamide group with protective and curative properties. It is absorbed through the leaves, stems and roots and acts by suppressing essential components of the disease so that the infection does not grow or spread (Anonymous 2023). The present study was carried out to assess fungicide sensitivity with respect to the status of *P. viticola* isolates, especially in vineyards where downy mildew was not under control even after several applications of different groups of fungicides.

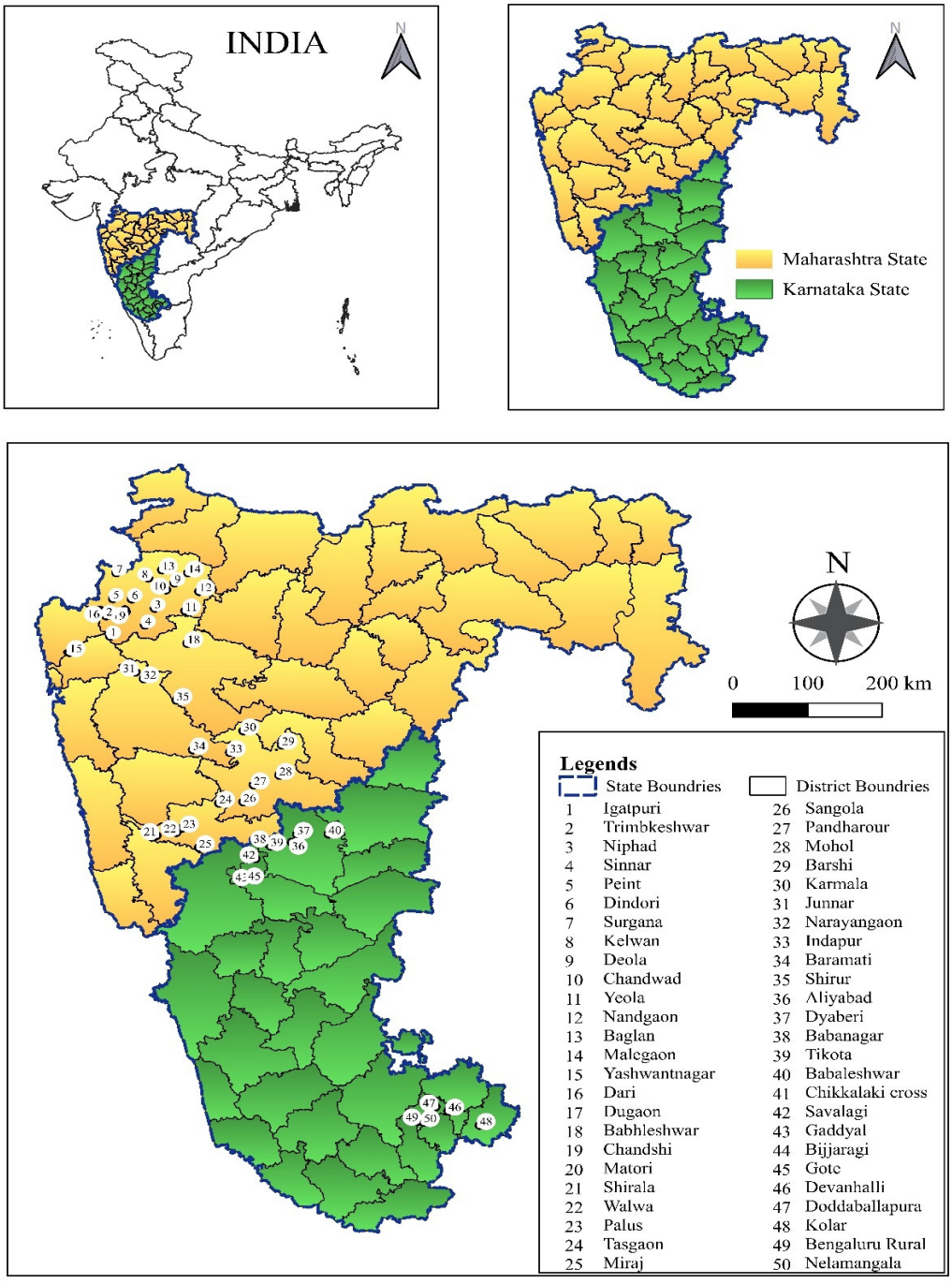

Fig. 1 : Collection of samples from Maharashtra state and Karnataka state

The experiment was conducted in the laboratory of the ICAR-NRC for Grapes Pune, India. Leaf samples infected with downy mildew were collected from vineyards from July 2020 to August 2021 (Fig. 1). The number of fields and total number of isolates collected from them (Table 1) were 50 and 500, respectively.

Maintenance of culture: Each isolate was maintained in triplicate on healthy Thompson Seedless plants under glasshouse conditions. Symptomatic leaves from these plants were used for further studies.

Material and Methods

Leaf disc assay method

The sensitivity of mefenoxam (manufactured by Syngenta India Limited, Pune) was determined via a modified 24-well leaf-disc bioassay method (Anonymous, 2023). Healthy leaves were taken from the 6th node from the apex of a growing shoot of the susceptible cultivar Thompson Seedless, and 15-mm disks were cut with the help of a sterile cork borer. The leaf discs were placed upside-down in wells containing 1 ml of 0.5% water agar solution. For fungicide application, the leaf discs were applied one day prior to inoculation with a high-volume low-pressure spray gun machine (10 µl per well). The concentrations used in the study were 0, 0.01, 0.1, 1, 3, 10 and 100 ppm. Each treatment was replicated 4 times. The discs treated with distilled water were used as controls. Leaf discs were inoculated with 10 µl of a suspension containing 50,000 sporangia/ml *P. viticola* collected from a single lesion on the maintained plates in the glasshouse. The plates were incubated at 22°C with alternating periods of 12 h of light and darkness. A binocular magnifier was used to quantify the lesion area following a seven-day incubation period. By comparing the treated and untreated control leaf discs, 100% of the infected leaf area was converted to the percentage of the infected leaf area. The sporulation of each inoculation point was rated via the following scale (Kraus *et al*., 2021):

0 = no sporulation

1 = light sporulation (difficult to see with the naked eye)

2 = sporulation area inferior to the diameter of the deposited inoculum droplet

3 = sporulation area corresponding to the diameter of the deposited inoculum droplet

4 = sporulation area greater than the diameter of the deposited inoculum droplet

The percent growth of the pathogen was calculated on the basis of sporulation at different concentrations. The highest concentration at which the isolates exhibited sporulation was also noted.

Table 1 : Locations and number of samples collected to study the sensitivity of *P. viticola to mefenoxam*

Locations	No of field for sample collection	No of leaf samples/field	No of single lesion isolates/20 leaves	Total no of isolates
Nashik	20	20	10	200
Sangli	5	20	10	50
Solapur	5	20	10	50
Pune	5	20	10	50
Bijapur	10	20	10	100
Bengaluru	5	20	10	50
Total samples for analysis	**50**	**-**	**-**	**500**

Result

Table 2 : Response of *P. viticola* to different concentrations of mefenoxam

Locations	Concentration (ppm) of Mefenoxam						
	0	**0.01**	**0.1**	**1**	**3**	**10**	**100**
Nashik	200	180	37	8	-	-	-
Sangli	50	50	14	2	-	-	-
Solapur	50	47	9	1			
Pune	50	42	20	7	-	-	-
Bijapur	100	95	18	4	-	-	-
Bengaluru	50	46	14	5	-	-	-
Total	**500**	**460**	**112**	**27**	-	-	-

Assessing the sensitivity of *Plasmopara viticola* to mefenoxam in different districts of Maharashtra

In the case of Nashik, Igatpuri smelting resulted in 2.11% fungal growth at 1 ppm (Fig. 2). In Trimbkeshwar tehsil, an average of four samples presented fungal growth at 0.1 ppm, i.e., 7.11% and 7.64% fungal growth was observed at the same concentration in Niphad tehsil. In Sinnar tehsil, maximum fungal growth was observed at 0.1 ppm, i.e., 8.04%. In the Peint samples, 1 ppm resulted in fungal growth, i.e., 4.95% growth. In Dindori, fungal growth of 2.63% was recorded for only one sample at 1 ppm. In Surgana, the average fungal growth of the two

samples was 2.48% at 1 ppm. In Kelwan, 7.41% of fungal growth was caused by a single isolate at 0.1 ppm. In the case of Deola, one sample presented a fungal growth of 5.14% at 1 ppm. In Chandwad, Yeola, Nandgaon and Baglan, the maximum fungal growth was observed at the 0.1 ppm concentration, i.e., 7.91%, 7.62%, 7.60% and 8.43%, respectively. In Malegaon, maximum fungal growth was also observed at 0.1 ppm, i.e., 7.01%. In the case of Yashwantnagar, the average fungal growth of the two isolates was 7.74% at the 0.1 ppm concentration. In Dari, 7.68% of fungal growth was caused by a single isolate at 0.1 ppm. A single isolate from Dugaon tehsil presented 1.13% fungal growth at 1 ppm. In Babhleshwar, the maximum fungal growth of Chandshi and Matori was observed at 0.1 ppm, i.e., 7.47%, 7.25% and 6.75%, respectively (Table 3).

In Sangli district, one isolate from Shirala tehsil presented 2.88% fungal growth at 1 ppm (Fig. 2), with corresponding 7.44% fungal growth at 0.1 ppm for the same isolate. In Walwa, the average of three samples presented fungal growth at 0.1 ppm, i.e., 7.19%. The sporulation of the pathogen was observed at 1 ppm (4.12%) of a single isolate at Palus. In Tasgaon and Miraj Tehsil, the average fungal growth of the three samples was 7.06 and 7.90, respectively, at 0.1 ppm (Table 3).

In the Solapur district, Sangola tehsil manifested greater average fungal growth at 0.1 ppm, i.e., 7.06%. (Fig. 2). In Pandharpur, sporulation of the pathogen was observed until 1 ppm (3.64%) of a single isolate was added. In Mohol, only one isolate manifested 6.95% fungal growth at 0.1 ppm. In Barshi and Karmala, the average fungal growth of the two isolates was 7.63% and 7.79%, respectively, at the 0.1 ppm concentration (Table 3).

Junnar tehsil of the Pune district recorded fungal growth of 3.66% at 1 ppm (Fig. 2). In Narayangaon, greater fungal growth was detected at 0.1 ppm, i.e., 7.21%. In Indapur and Baramati, the maximum fungal growth at 1 ppm was 2.44% and 2.79%, respectively. In Shirur tehsil, one isolate also grew at 1 ppm, i.e., 5.01%, with corresponding growth rates of 8.36% at 0.1 ppm and 17.03% and 13.24% at 0.01 ppm (Table 3).

Table 3 : Average growth sensitivity of *P. viticola* to mefenoxam in the Nashik, Sangli, Solapur and Pune districts of Maharashtra.

District	Tehsil/village	Average of Fungal Growth (sporulation) compared to untreated control (%)						
		0 ppm	0.01 ppm	0.1 ppm	1 ppm	3 ppm	10 ppm	100 ppm
Nashik	Igatpuri	35.70	11.50	7.21	2.11	0.00	0.00	0.00
	Trimbkeshwar	39.70	14.132	7.11	0.00	0.00	0.00	0.00
	Niphad	46.25	14.41	7.64	0.00	0.00	0.00	0.00
	Sinnar	40.94	14.42	8.04	0.00	0.00	0.00	0.00
	Peint	37.06	15.67	6.15	4.95	0.00	0.00	0.00
	Dindori	31.26	14.16	6.54	2.63	0.00	0.00	0.00
	Surgana	35.88	12.845	7.03	2.48	0.00	0.00	0.00
	Kelwan	42.55	12.84	7.41	0.00	0.00	0.00	0.00
	Deola	41.00	12.03	7.02	5.14	0.00	0.00	0.00
	Chandwad	45.36	15.40	7.91	0.00	0.00	0.00	0.00
	Yeola	35.685	13.35	7.62	0.00	0.00	0.00	0.00
	Nandgaon	41.55	15.63	7.60	0.00	0.00	0.00	0.00
	Baglan	35.56	13.66	8.43	0.00	0.00	0.00	0.00
	Malegaon	32.67	15.42	7.01	0.00	0.00	0.00	0.00
	Yashwantnagar	41.17	12.98	7.74	0.00	0.00	0.00	0.00
	Dari	46.94	14.84	7.68	0.00	0.00	0.00	0.00
	Dugaon	37.16	14.98	8.36	1.13	0.00	0.00	0.00
	Babhleshwar	40.31	12.40	7.47	0.00	0.00	0.00	0.00
	Chandshi	39.14	15.3	7.25	0.00	0.00	0.00	0.00
	Matori	37.71	13.80	6.75	0.00	0.00	0.00	0.00
Solapur	Sangola	34.93	12.65	7.06	0.00	0.00	0.00	0.00
	Pandharpur	31.75	17.36	6.95	3.64	0.00	0.00	0.00
	Mohol	31.02	16.77	6.95	0.00	0.00	0.00	0.00
	Barshi	34.47	16.96	7.63	0.00	0.00	0.00	0.00
	Karmala	38.81	13.01	7.79	0.00	0.00	0.00	0.00

Sangli	Shirala	38.77	12.35	7.44	2.88	0.00	0.00	0.00
	Walwa	37.30	16.12	7.19	0.00	0.00	0.00	0.00
	Palus	38.94	11.69	8.01	4.12	0.00	0.00	0.00
	Tasgaon	40.00	15.08	7.06	0.00	0.00	0.00	0.00
	Miraj	37.58	11.95	7.90	0.00	0.00	0.00	0.00
Pune	Junnar	36.2	12.02	7.41	3.66	0.00	0.00	0.00
	Narayangaon	38.96	12.62	7.21	0.00	0.00	0.00	0.00
	Indapur	40.86	14.12	7.60	2.44	0.00	0.00	0.00
	Baramati	35.21	14.20	7.06	2.79	0.00	0.00	0.00
	Shirur	34.19	17.03	8.36	5.01	0.00	0.00	0.00

Assessing the sensitivity of *Plasmopara viticola* to mefenoxam in different districts of Karnataka

In the Bijapur district, at Aliyabad tehsil, the average fungal growth of the two samples was 6.49% at 0.1 ppm (Fig. 2). In Dyaberi, the maximum fungal growth of 8.51% was observed at 0.1 ppm. In Babanagar, at a 1 ppm concentration, growth was 1.45%. In Tikota, maximum fungal growth was also recorded at 1 ppm, i.e., 4.43%. In Babaleshwar, the 1 ppm concentration resulted in 2.4% fungal growth, and at the 0.1 ppm concentration, 8.65% and 7.12% fungal growth were observed. In Chikkalaki Cross and Savalagi, only the 0.1 ppm concentration resulted in fungal growth. In Gaddyal, the pathogen was responsive to 1 ppm, with a growth of 2.89%. In Gote and Bijjaragi, fungal growth was observed only at 0.1 ppm (Table 4).

In Devanhalli tehsil in the Bengaluru district, the average fungal growth of the three isolates was 8.29% at 0.1 ppm (Fig. 2). One isolate from Doddaballapura exhibited 2.84% fungal growth at 1 ppm, whereas four samples were sensitive until 0.1 ppm. At the Kolar concentration, sporulation of the pathogen was observed at 1 ppm (3.69%) of a single isolate, whereas the sporulation of the other isolate was 8.54% at 0.1 ppm. In Bengaluru Rural Brazil, maximum fungal growth was observed at 1 ppm (1.25%). In Nelamangala, two isolates manifested an average fungal growth of 4.56% at a 1 ppm concentration, whereas the average fungal growth of three isolates was 0.1 ppm. There was no growth of *Plasmopara viticola* at concentrations of 3, 10 and 100 ppm at any of the locations (Table 4).

Table 4 : Average growth sensitivity of *P. viticola* to mefenoxam in the Bijapur and Bengaluru districts of Karnataka.

District	**Tehsil/village**	**Average of Fungal Growth (sporulation) compared to untreated control (%)**						
		0 ppm	0.01 ppm	0.1 ppm	1 ppm	3 ppm	10 ppm	100 ppm
Biapur	Aliyabad	41.48	14.80	6.49	0.00	0.00	0.00	0.00
	Dyaberi	43.18	15.23	8.51	0.00	0.00	0.00	0.00
	Babanagar	31.06	16.74	8.36	1.45	0.00	0.00	0.00
	Tikota	34.25	16.22	8.32	4.43	0.00	0.00	0.00
	Babaleshwar	42.5	12.02	8.65	2.40	0.00	0.00	0.00
	Chikkalaki Cross	32.51	13.36	6.98	0.00	0.00	0.00	0.00
	Savalagi	22.07	13.56	7.80	0.00	0.00	0.00	0.00
	Gaddyal	42.65	13.03	7.41	2.89	0.00	0.00	0.00
	Bijjaragi	33.00	12.61	7.31	0.00	0.00	0.00	0.00
	Gote	41.45	14.02	8.66	0.00	0.00	0.00	0.00
Bengaluru	Devanhalli	39.13	13.64	8.29	0.00	0.00	0.00	0.00
	Doddaballapura	36.98	16.27	7.28	2.84	0.00	0.00	0.00
	Kolar	30.12	15.74	9.00	3.69	0.00	0.00	0.00
	Bengaluru Rural	40.99	13.74	7.65	1.25	0.00	0.00	0.00
	Nelamangala	51.79	14.44	7.57	4.56	0.00	0.00	0.00

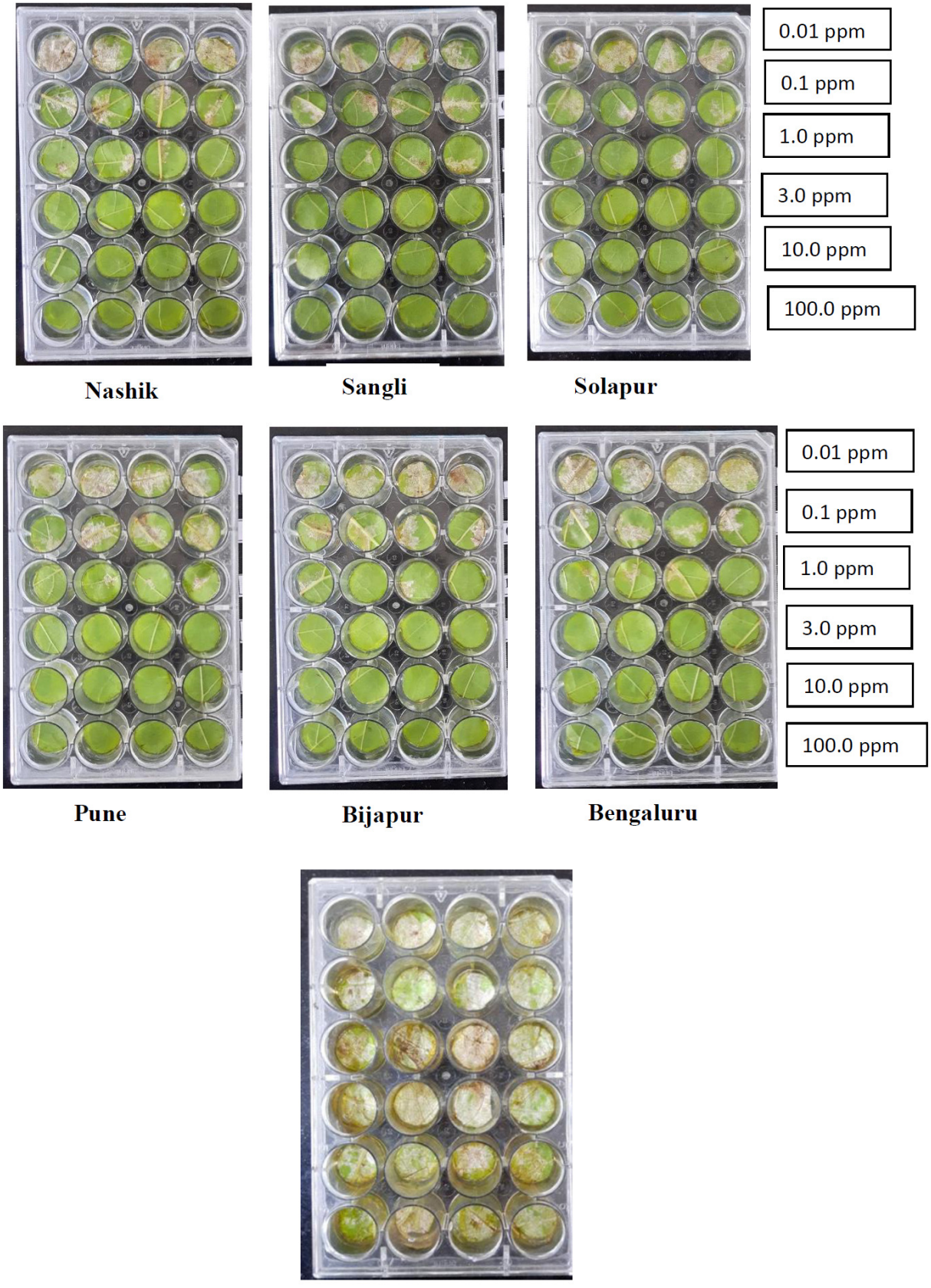

Fig. 2 : In vitro response of *Plasmopara viticola* to different concentrations of Mefenexom

Discussion

Among the 500 samples, 348 samples showed growth at 0.01 ppm, 85 samples at 0.1 ppm and 27 samples at 1 ppm. Forty samples did not show any symptoms at any of the concentrations. The data revealed that *Plasmopara viticola* was slightly resistant to Mefenoxam. Mefenoxam is classified as a high risk of developing resistance by the Fungicide Resistance Action Committee (FRAC) (FRAC 2015) because it inhibits the target pathogen's ribosomal RNA polymerase. Colcol and Baudoin (2016) reported that there was no resistance to Mefenoxam among isolates of *Plasmopara viticola* in the USA. The mechanism conferring resistance to mefenoxam has not been elucidated but may involve a major gene and several minor genes (Blum and Gisi, 2008; Gisi and Sierotzki, 2008a).

However, in France, *P. viticola* populations are sensitive, resistant and highly resistant to the phenyl amide group of fungicides, i.e., metalaxyl has been reported on the basis of MIC values of <10, 100, and ≥ 200 µg mL^{-1} (Fourie, 2004). In the case of another oomycetous pathogen, *Phytophthora infestans,* a sensitive isolate became tolerant after a single passage on mefenoxam-containing medium (Childers *et al*., 2015). Research on strand-specific RNA sequencing revealed that several genes were upregulated and responsible for acquired resistance. A combination of computational analysis and experimental approaches was used to identify differentially expressed genes with a potential association with the phenomenon (Gonzalez–Tobon *et al*., 2022). Similar studies may also be carried out in the case of mefenoxam to understand its sensitivity at 1 ppm.

The resistance of Plasmopara viticola to Mefenoxam in all grape-growing regions suggests that a premix of this fungicide with a contact fungicide is needed for more effective control of the downy mildew of grapes. Compared with oxathiapiprolin alone, the combination of oxathiapiprolin and mefenoxam demonstrated equally effective performance against *Pseudoperonospora cubensis*, causing downy mildew in cucumber. As stated by Cohen *et al*. (2018), the combination of oxathiapiprolin and amisulbrom (Pharate *et al*., 2023), the combination of oxathiapiprolin and Mancozeb (Phad *et al*., 2023) and oxathiapiprolin + mandipropamid (Saha *et al*., 2024) demonstrated superior efficacy against the downy mildew of grapes. They also reported similar findings, emphasizing that the effectiveness and interaction of two fungicides in combination depend on their distinct mechanisms of action.

Conclusion

Among the 500 samples, 69.6% of the samples exhibited growth at 0.01 ppm, 17% of the samples were symptomatic at 0.1 ppm, 5.4% of the samples presented symptoms at 1 ppm, and 8% of the samples did not show any symptoms at any of the concentrations. The resistance of Plasmopara viticola to Mefenoxam in all grape-growing regions could be a major problem in mitigating this disease, and premixes need to be developed for desirable and sustainable management of the downy mildew of grapes.

Competing Interests: The authors declare no potential conflicts of interest.

Author Contributions: This work was carried out in collaboration with all the authors. Authors SS and SP planned the research work. Author SP carried out a lab study and collection of samples. 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.

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