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Novel approach of validation of online interactive weather information-based disease risk assessment in grapes in India

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Grapevine (*Vitis vinifera)* is a globally important horticultural crop cultivated all over the world for its myriad uses. It is subjected to the infection of several diseases which attack susceptible grapevine varieties and cause severe loss in yield as well as to the economy. Application of fungicides is the major weapon against plant diseases, however, continuous application of fungicides leads to environmental pollution, emergence of fungicide resistance and pesticide residue in grapes. A weather-based disease forecasting model would enable the growers to take decisions at the right time for fungicide spray which will help in better disease management with reduced fungicide use. A reasonable level of prediction of downy mildew was obtained by using the disease forecasting model of MatWin II and iMETOS automatic weather data recorded. A multi-location trial was initiated to validate the prediction system to check the prediction of downy and powdery mildew and total reduction in the number of fungicide sprays for three consecutive years. Sprays were suggested when risk of disease was indicated by the model. Total five major parameters were observed *i.e.,* terminal PDI of powdery and downy mildew, reduction in the number of sprays, yield and number of pesticide detection in both the plots. Results showed the significant reduction in terminal PDI of downy and powdery mildew along with increased yield and reduction in number of sprays and pesticide detections.

Keywords: Disease forecasting model, Downy mildew, Grapes, Powdery mildew, Prediction system,

INTRODUCTION

Grapevine (*Vitis vinifera)* is a globally important horticultural crop cultivated all over the world. It is the fifth most widely cultivated fruit after banana, watermelon, apples and citrus in the world (Anonymous 2021). The grape itself is used for a myriad of products, ranging from fresh fruit, preserves, juice, wine and raisins. It is a rich source of nutrients like minerals, vitamins, antioxidants like resveratrol, etc. The major medicinal properties of grape and its constituents include antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, anti-atherogenic, neuroprotective, anti-obesity, anti-aging and anti-infection attributes (Yadav *et al*. 2009). In India a production of 3213 Thousand MT from an average area of 152 Thousand ha in the year 2020-21 was recorded (Anonymous, 2022). However, grapevine is subjected to the infection of several diseases which attack susceptible grapevine varieties and cause severe loss in yield as well as to the economy (Mannini

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and Digiaro 2017). The most significant grapevine diseases are Anthracnose (c.o. *Colletotrichum gloeosporioides*), Downy mildew (*Plasmopara viticola*), Powdery mildew (*Erysiphe necator*) and Bacterial spot (*Xanthomonas campestris* pv. *viticola*).

Application of fungicides is the major weapon in the arsenal of plant pathologist to counter the onslaught of diseases. However, incessant application of fungicides not only aggregate the environmental pollution but also results in emergence of fungicide resistance and pesticide residue in grapes. Grape being an export crop from India, pesticide residues in the produce is a serious concern and can affect the national exchequer adversely. As it is a known fact that disease incidence and severity is dependent on weather parameters (Jamadar and Sataraddi, 2010) a weather-based disease forecasting model would enable the growers to take decisions at the right time for fungicide spray which will help in better disease management with reduced fungicide use. An online interactive model with the weather parameters like temperature, rainfall, leaf wetness, relative humidity etc. were recorded at regular short intervals and the disease risk was predicted through the use of a software at ICAR-National Research Centre for Grapes, Pune (Sawant and Adsule, 2011). A reasonable level of prediction of downy mildew was obtained by using the disease forecasting model of MatWin II and iMETOS automatic weather data recorded (Sawant and Sawant, 2012). However, the prediction system needed to be validated in all the grape growing areas and hence, the trial was envisaged to check not only the prediction of downy and powdery mildew, but also observe the total reduction in the number of fungicide sprays.

MATERIAL AND METHODS

The trial was conducted in five locations; (i) Grapes Research Station, Rajendranagar, Telangana, (ii) Grapes Research Station, Theni, Tamil Nadu, (iii) Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, (iv) College of Horticulture, Mandsaur, Madhya Pradesh (v) Horticulture Research and Extension Centre, Vijayapura, Karnataka for three consecutive years *viz.* 2018, 2019 and 2020. Two vineyards of 0.5 acre each was considered for study in each location. Vineyard I was managed for powdery and downy mildew diseases based on online advisory (Advisory plot/AP) while in vineyard II the spray schedule of farmer (Farmer plot/FP) was followed. At each location an iMETOSj Automatic Weather Station (AWS) with temperature, relative humidity and leaf wetness sensors was installed. Disease management guidelines at various levels of risk estimated by the model and growth stages have been prepared for Maharashtra conditions. Sprays are suggested when risk of disease is indicated by the model. Water volume used for spray was calculated based on requirement of 1000 L/ha at full canopy. Knapsack sprayer fitted with hollow cone nozzle was used for spray. Disease severity was rated on a 0 to 4 scale (where 0= No disease present, 1= 15-25% leaf area and berries infected, 2=26%-50% leaf area and berries infected, 3= 51%-75% leaf area and berries infected, 4= more than 75% leaf area and berries infected) (Horsfall and Heuberger 1942).

Terminal percent disease index (PDI) was determined according to the formula (McKinney 1923)

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

Terminal Residue detection:

Apparatus

The certified reference materials (CRMs) and samples were weighed using a precision analytical balance (Model Vibra and 420 ADJ, Adair Dutt, Mumbai, India). At various phases of sample preparation, a heavy-duty mixer/grinder (Vishvakarma Machine Tools, Rajkot, India), a high-speed homogenizer (DIAX-900, Heidolph, Schwabach, Germany), a high-speed centrifuge (Kubota Corp., Tokyo, Japan), a low-volume concentrator (TurboVap® LV, Caliper Life Science, USA), and a micro-centrifuge (Microfuge Pico, Kendro, D-93 37520, Osterode, Germany) were utilized.

Chemicals and reagents

The CRMs of purity 98-99.9% was procured from Dr Ehrenstorfer GmbH (Augsburg, Germany).

However, Merck (Darmstadt, Germany) provided the mass spectrometry-grade extraction solvents and other reagents, including acetonitrile, ethyl acetate, methanol, water, and acetic acid.

Preparation of standard solutions

Individual pesticide stock solutions were made by dissolving 10 (±0.01) mg of each certified reference materials (CRM) in 10 mL of solvent (Ethyl acetate/Methanol). Using appropriate dilutions, intermediate standard mixtures of 10 mg/kg and working standard mixtures of 1 mg/kg were prepared by diluting in the solvent. The calibration standards for GC-MS/MS were prepared by serial dilution with ethyl acetate at concentrations ranging from 0.005-0.250 mg/kg and for LC-MS/MS; standard solutions ranging from 0.001 to 0.05 mg/kg were prepared in a methanol: water mixture (50:50). The matrix-matched calibration standards were prepared by using the control matrix extracts.

Sample preparation

The entire laboratory sample was thoroughly crushed in a mixer and grinder, and approximately 200 g of it was homogenized to a smooth paste. In a 50 mL polypropylene centrifuge tube, a sample (10 g) was drawn and 10 mL ethyl acetate and 10 g anhydrous sodium sulphate were added. The mixture was homogenised for 2 minutes before being centrifuged at 5000 rpm for 5 minutes. By vigorously shaking for 30 seconds, an aliquot of the supernatant ethyl acetate layer (5 mL) was cleaned by dSPE with PSA (25 mg). The 2 ml extract was then evaporated and reconstituted in a 1:1 methanol and water solution containing 0.1% acetic acid, and the extract was filtered using 0.2 nylon membrane filter paper before being injected into an LC-MS/MS. Similarly, 1 mL of supernatant ethyl acetate layer was cleaned by dSPE with PSA (25 mg) by vigorously shaking for 30 seconds, filtered through PTFE, and injected into GC-MS/MS for analysis.

LC-MS/MS for multiresidue analysis

The target analytes were separated chromatographically using a Restek column (3 ìm, 100 mm length, 2.1 mm ID; Restek Corporation, Bellefonte, USA). Sciex liquid chromatography was coupled to a Sciex QTRAP 5500 mass spectrometer in the LC-MS/MS system (Toronto, Canada). Analyst 1.7.1 software was used to execute the test. The mobile phase consisted of 10 mM ammonium formate + 0.1 percent formic acid in (A) water and (B) methanol. The total run time was 20 minutes with a 0.5 mL/min flow rate, and the injection volume was 10 µL. The temperature in the column oven was kept at 40 (1°C). The gradient profile was 0-2.5 min, 95% A; 2.5-5 min, 40% A; decreasing to 5% A; 5-12.5 min, 5% A; 12.5-16.5 min, 2% A increasing to 95% A; and 16.5-20 min, 95% A.

The residue concentration measurements were done with electrospray ionization (ESI) in positive polarity with optimized multiple reaction monitoring (MRM) transitions. The source parameters, namely, ion source voltage (5500 V), nebulizer gas (30 psi), heater gas (60 psi), ion source temperature (550 °C), and curtain gas (40 psi), were maintained throughout the analysis.

The pesticide residues estimation was performed by a retention time dependent scheduled multiple reaction monitoring' (sMRM) with two mass transitions for each test pesticide; one for quantification and the other for qualitative confirmation, all within a 30 percent ion ratio variation tolerance limit. With a target scan time of 2 second, the MRM detection window was set at 90 seconds.

GC-MS/MS multiresidue analysis

The analysis was performed using Agilent GC (7890A) equipped with a CTC Combipal (CTC Analytics, Switzerland) auto sampler, connected to a triple quadrupole mass spectrometer (7000B, Agilent Technologies, Santa Clara, USA)with Mass Hunter software (ver. B.05.00.412). The programmable temperature vaporisation (PTV) was operated in splitless mode, and 1µL of the sample was injected into a PTV Siltek Metal Liner (2mm x 2.75mm x 120m). TG-5MS (5% phenylmethylpolysiloxane; Thermo Scientific, USA) capillary columns (30 m × 0.25 mm, 0.25 µm) Ultra-pure grade helium (BOC India Ltd., Kolkata) was used as the carrier gas with constant

1.2 mL/min flow. The injector program started at 80 ! (0.1 min hold), then further at 14.5 °C/s to 290 ! (0.5 min hold), followed by rapid heating at 300 °C (10 min hold). Oven temperature was programmed with the initial temperature at 90 °C (1 min hold); ramped at 40 °C min^{-1} to 170 °C (0 min hold), then at 15 °C min^{-1} up to 290 °C with hold for 6 min resulting in a total run time of 21 min. The temperature levels of the ion source, and transfer line were set at 230 °C, and 290 °C, respectively.

Reduction in the number of sprays was calculated by deducting the number of sprays applied in AP from the number of sprays applied in FP. The marketable yield was harvested and extrapolated to tonnes/Ha.

RESULTS AND DISCUSSION

In the course of three years, five major parameters were observed *i.e.* terminal PDI of powdery and downy mildew, reduction in the number of sprays, yield and number of pesticide detection in both the plots. In all the locations terminal PDI of powdery mildew was lower in case of AP than FP (Table 1). At the end of third year, minimum PDI was observed in Rajendranagar *i.e.* 3.5 followed by Rahuri location with 5.28 in AP. The PDI was maximum in both AP and FP Mandsaur centre, in all the three years of study. In case of terminal PDI of downy mildew (Table 2), Mandsaur location did not have any incidence of disease in the first two years, but in the third year disease incidence was highest in FP *i.e.* 35.6 among all the locations. Minimum PDI of 2.58 at the end of the third year was observed in Rahuri followed by Vijayapura which recorded a terminal PDI of 4.9.

The yield data clearly expressed the superiority of AP as compared to FP, in all the three years under study (Table 3**).** Highest yield was obtained at Rajendranagar location during 2020 in AP *i.e.* 32.46 t/ha. The increase in yield in AP was achievable even though the number of sprays in all AP plots were reduced (Table 4). In Mandsaur location during the first two years, there was only a reduction of two sprays, but in the third year, there was a reduction of six sprays. In Vijayapura location, the number of sprays reduction remained same in the first two years *i.e.* 10 sprays, but it was increased to 24 in the third year.

Reduction in the number of sprays is reflected by the number of pesticide detected in the different locations in multi residue analysis (Table 5) done by LC-MS/MS and GC-MS/MS. Rajendranagar location had a consistent fewer detection in AP than FP. At the end of third year, Vijayapura centre had the least detection in AP *i.e.* three as compared to FP which manifested 11 detections. Mandsaur at the end of third year had no detection in AP while 6 detections were reported in FP.

Comparative evaluation of pesticide detection in the farmers practice plot and technology plots revealed that the total number of detections in the advisory plot was less as compared to farmers plot. All the detected fungicides were below the standard EU MRL mentioned in Annexure 5 available on ICAR-National Research Centre for Grapes, Pune website (Anonymous 2023) During the three years of study, total of 10 fungicides were detected in the farmer's plot, whereas only 4 detections were observed in the advisory plot at Rahuri centre (Fig. 1). In 2020, no fungicide detections were observed in the advisory plot however 5 detections were observed in farmers practice plot. At Vijayapura centre, the fungicide detections were observed to be increased from 6 to 11 in farmer's plot, whereas, the technology plot showed a gradual decrease in fungicide detections from 5 to 3 (Fig. 2). All the detected fungicides residue were below the EU MRL. At Mandsaur centre, the same trend was observed for fungicide detections as Vijayapura. After 2019, no fungicide detections were observed in advisory plots (Fig.3). At Rajendranagar, fungicide detections were increased year wise in farmers practice plot. In advisory plots during first two years 5 detections were observed and in the third year only 3 detections were observed (Fig.4). All the detections were below the EU MRL.

Epidemiological studies on grape diseases had been reported on anthracnose from India (Ghule *et al*. 2015) based on rainfall, temperature and relative humidity, but a system based approach

Table 1. Comparative data on terminal PDI of powdery mildew in farmers plot and advisory plot

Name of centers	Terminal PDI (Powdery mildew)					
	2018		2019		2020	
	FP	AP	FP	AP	FP	AP
Rahuri	6.49	6.34	11.50	9.20	8.45	5.28
Vijayapura	20.45	2.50	21.50	19.20	7.20	6.30
Mandsaur	38.37	21.00	37.60	21.40	35.30	21.80
Rajendranagar	6.75	3.25	5.80	3.80	6.75	3.50
Periyakulam	17.08	9.76	16.30	26.20	29.20	10.70

*FP: Farmer plot, AP: Advisory plot

Table 2 : Comparative data on terminal PDI of downy mildew in farmers plot and advisory plot

Name of centers	Terminal PDI (Downy mildew)					
	2018		2019		2020	
	FP	AP	FP	AP	FP	AP
Rahuri	10.26	5.90	8.70	4.80	5.38	2.58
Vijayapura	14.25	11.35	16.90	15.30	18.70	4.90
Mandsaur	Nil	Nil	Nil	Nil	35.60	29.10
Rajendranagar	1.75	3.75	2.30	1.50	5.25	2.50
Periyakulam	7.36	1.38	8.36	1.18	12.60	5.60

*FP: Farmer plot, AP: Advisory plot

Table 3 : Comparative data on yield of grapevine in farmers plot and advisory plot

Name of centers	Yield (t/Ha)					
	2018		2019		2020	
	FP	AP	FP	AP	FP	AP
Rahuri	19.12	22.76	18.12	21.36	17.11	19.62
Vijayapura	21.7	22.61	21.7	22.63	23.4	25.03
Mandsaur	16.00	18.00	15.80	17.50	14.80	15.35
Rajendranagar	28.45	30.89	27.15	31.03	30.25	32.46
Periyakulam	19.22	20.73	18.12	20.73	17.63	19.82

*FP: Farmer plot, AP: Advisory plot

Table. 4: Reduction in number of sprays of pesticides

Name of centers	No. of Sprays (PM+DM)								
	2018			2019			2020		
	FP	AP	Reduction in Sprays	FP	AP	Reduction in Sprays	FP	AP	Reduction in Sprays
Rahuri	44	26	18	32	17	15	28	16	12
Vijayapura	36	26	10	36	26	10	52	28	24
Mandsaur	8	6	02	8	6	02	7	13	06
Rajendranagar	17	11	06	17	12	05	16	11	05
Periyakulam	35	24	11	47	31	16	17	26	09

*FP: Farmer plot, AP: Advisory plot

Table 5: Comparative data on residue analysis of grapes in farmers plot and advisory plot

Name of centers	No. of pesticides detected					
	2018		2019		2020	
	FP	AP	FP	AP	FP	AP
Rahuri	11	2	9	4	-	-
Vijayapur	6	5	-	-	11	3
Mandsaur	-	-	3	Nil	6	Nil
Rajendranagar	8	6	9	6	10	8
Periyakulam	-	-	-	-	-	-

*FP: Farmer plot, AP: Advisory plot

to control powdery and downy mildew was lacking in the multi locational domain. Eswari (2021) had reported a PDI model in grapes with report to downy mildew incidence, yield and climatic variables from Tamil Nadu, but the parameter of pesticide application and detection of residues was not included in the study which was covered in the present investigation. Timing of fungicide application in horticulture crop has traditionally relied on strict calendar based methods that results in re application intervals ranging from 7 to 28 days. To time fungicide applications to prevent disease outbreaks, weather dependent predictive algorithms have been developed using risk indices or linear regression techniques to prevent infection periods in numerous horticultural crops like spinach (Sullivon *et al.*, 2003) and carrots (Rogers and Steveason, 2006). Leaf-based infection models to guide fungicide application in downy mildew had been developed by Caffi*et al.* (2009, 2011) and was further improved by Brischetto *et al.* (2021) taking into consideration that secondary infection cycles are more important than primary cycles late in the season. The present study not only predicted the incidence of diseases via the AWS, but also guided the timing of spray which in turn reduced the frequency of fungicide spray vis-à-vis the total number of detections in the season. The entire mechanism had been recommended in all the grape growing areas via the All India Coordinated Research Programme (fruit crops) and have recognized as a technology generated (Patil *et al.*2021)

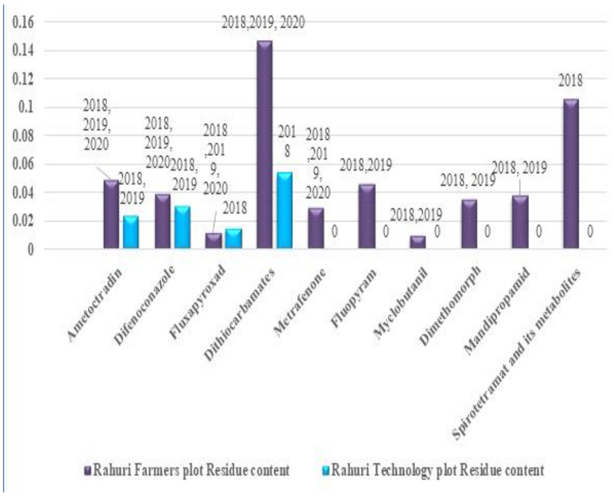

Fig.1: Fungicide residue detected in farmers plot and advisory plot at Rahuri Centre

Fig. 2: Fungicide residue detected in farmers plot and advisory at Vijayapura Centre

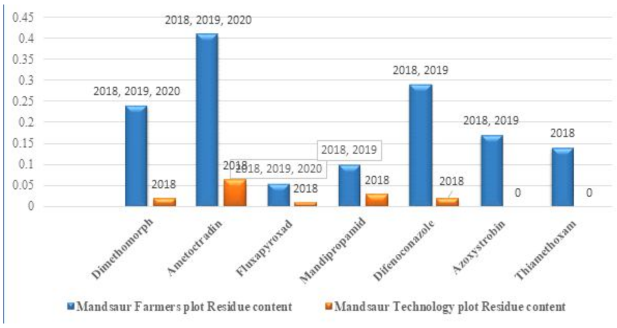

Fig.3: Fungicide residue detected in farmers plot and technology plot at Mandsaur Centre

The disease prediction model helped in reducing theterminal PDI of powdery and downy mildew with the reduction in the number of fungicide sprays. The increase in yield was observed in advisory plot as compared to farmers practise plot. Application of this model, number of pesticide detections were minimized. Among the number of disease prediction model, this model was found to be widely accepted among the farmers. However, periodic validation needs to

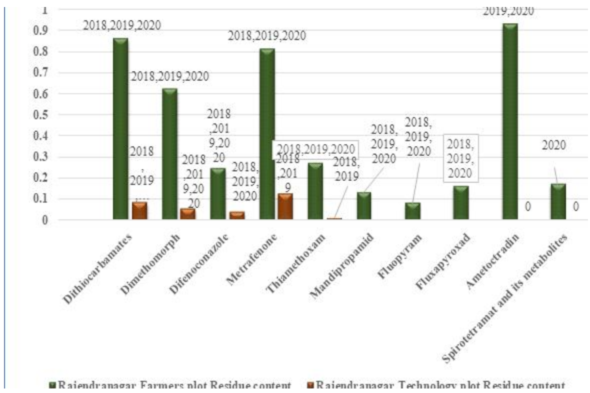

Fig.4 :Fungicide residue detected in farmers plot and advisory plot at Rajendranagar Centre

be done to check its efficacy as climate change is playing a vital role in disease development and host-pathogen interaction.

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