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Pesticide use trends in vineyards and at harvest when need base application is done

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Abstract
Twenty four progressive growers were surveyed for understanding the standard disease management practice followed by them and common pesticide residues encountered. Grapes from each vineyard were sampled at harvest using the protocol specified by APEDA –National Referral Laboratory, Pune and multi-residue analysis was performed on LC-MS/MS and GC-MS/MS. Out of 21 farmers only 7 had used biocontrol agents, and numbers of sprays were in the range of 1-3 for diseases, while 1-8 for insect control. Residue analysis indicated that majority of pesticides detected below MRLS, only residues of powdery mildew fungicides, flusilazole and hexaconazole were detected above MRL in 1 and 3 cases respectively. Therefore, application of Triazole fungicides were considered unsafe after 60 days of fruit pruning. In most cases fungicides applied before fruit set were not detected in residue, except that CAA fungicides (dimethomorph, mandipropamid and iprovalicarp) were detected even when applied at 40 days of pruning. CAA fungicides were thus considered safe only during flowering phase. Cymoxanil a fungicide for downy mildew was not detected even when applied up to 60 days of pruning and hence considered safe for application when downy mildew control is needed after fruit- set. Potassium salt of phosphoric acid in combination with mancozeb was safe up to 60 DAP.

Keywords: Grapes, pesticide use trend, pesticide residues, *Vitis vinifera*, food safety

Introduction

In India, more than 3/4 of the grape area lies in the state of Maharashtra which accounts for eighty percent of total production (NHB, 2017) [20]. Majorly five to six *Vitis vinifera* table grape cultivars are grown and all of them are highly prone to diseases and insect pest attack almost throughout the year. This necessitates frequent use of pesticides in vineyards as preventative and curative applications. Incidence of powdery mildew and attack of insect pests like thrips, mealy bugs and mites can occur even near harvest (Sawant and Sawant, 2012; Yadav and Amala, 2013) [16, 19] and growers have no alternative other than pesticide application to prevent loss of yield and quality of fruits. A number of pesticides are registered for management of these diseases and pests (APEDA, 2016a) [3] with guidelines on their use in vineyards, especially the pre-harvest intervals (PHIs) to be maintained to avoid presence of objectionable levels of residues at harvest. In many instance frequent late pesticide applications results in presence of terminal pesticide residues above the legally specified levels by the country of destination and this has become a main trade obstacle for the stakeholders of the grape industry. Any detection of pesticides in exportable commodities by the importing countries results in rejection of exported consignments and causes huge financial loss to the grape growers.

To avoid rejections by importing countries, mainly the European Union, at the port of import, a monitoring program for pesticides residues in grapes is implemented in India since 2003-2004. The MRL non-compliance of fresh grapes for export to the European Union countries has reduced from 23.69% in 2003-2004 to 7.16% in 2005-2006 (Anonymous, 2008) [1]. The main aim was not only to reduce number of sprays required but also to improve the efficiency of pest management program by selecting the appropriate pesticide and timing of application. A recent study has shown that Centre's intervention had played a significant role in promoting GAP for grapes (Som *et al*., 2016) [18]. Currently, on an average 18000 farms (equivalent to 18000 ha) are registered for export under the Grapenet grogram of APEDA. This brought out the need for analysis of the constraints faced by these farmers. The present study was thus, carried out to review the pest management practices followed and their terminal residue encounter at harvest so that future interventions can be planned for production of residue free grapes.

Material and Methods

Selection of vineyards

Twenty four *Vitis vinifera* table grape vineyards belonging is enlightened growers who are in contact to and hence the chances of adherence to GAP was expected and detection of pesticide residues were minimal were selected. Successful farmers who are in grapes trade from longtime and changed need base sprayed instead of schedule based. Surveyed vineyard analysed for whether we follow their schedule as it is or some fine tuning are required. These farmers groups selected were educated earlier by RMP and make effort to reduce pesticides residue and produce quality grapes for export to other country other than EU or local market. These vineyards were located in Pune, Solapur, Nasik and Sangli regions of Maharashtra, India. Grape samples were collected at their optimum maturity during the month of February to March, 2017.

Pesticide use trend in vineyards

The pesticides used during the fruiting growth season in each of the twenty one vineyards were obtained from the daily diary maintained by the growers. The PHI and maximum residue limit (MRL) set by European Union were taken from Annexure-5 of the document "Procedures for export of fresh table grapes to the European union" (APEDA, 2016a) [3].

Collection of grape samples

Sampling of grapes for analysis of pesticide residues was performed as per recommended protocol as per Annexure-7 (APEDA, 2016b) [4]. A bunchlet comprising of 6-8 berries from the lower portion of healthy bunches that had attained optimum maturity, size, and colour were collected from randomly selected vines at different spots in the vineyard. Two representative samples, each of two kg weight, was boxed, labeled and transported to the laboratory within 8 hrs of sampling and stored at 0 ºC until processed.

Multi- pesticide residue analysis

The samples were extracted by ethyl acetate and cleaned by dispersive solid phase extraction with PSA the method reported earlier (Banerjee *et al*., 2012) [6]. The residues were analyzed by GC-MS/MS (7890GC-7000C-MS) using a gas chromatograph (Agilent 7890A, Agilent Technologies, Palo Alto, USA) coupled to an electron impact ionization triple quadrupole mass spectrometer (Agilent, 7000C, Agilent Technologies, Palo Alto, USA) and also by liquid chromatography−tandem mass spectrometry (LC-MS/MS)(API-5500, AB Sciex, Toronto, Canada)) (Jadhav *et al*., 2015) [9]. The GC-MS/MS method covered 375 test analytics and LC-MS/MS method covered 173 analytics with an acceptable limit of quantification (LOQ) of <10 ppb per >90% of analytics. The methods covered all the pesticides recommended in grape as per Annexure-5 and list of chemicals monitored in grapes for export purpose as per Annexure-9 of the document "Procedures for export of fresh table grapes to the European union" (APEDA, 2016c) [5]. Five points matrices match calibration in the range of 0.005 → 0.1000 mg/kg were used for quantification of residue. The average recovery evaluated at two spicing level (0.010 and 0.05 m/kg) offered an accepted accuracy within the range of 70-120% with RSD < 20%.

Multi-residue method for simultaneous estimation of CPPU, 6-BA, GA3 and ethephon residues in grapes (PGRs).

Sample preparation

The grapes berries 200 g, crushed in a blender were homogenised carefully, from homogenized sample take 10 g of sample, added to a 50 mL centrifuge tube. The sample was fortified with 0.1 mg/kg Ethephon. The sample was extracted with 20 mL of methanol (acidified) containing 1% formic acid. Draw 0.5 mL supernatant and dilute with 0.5 mL methanol and then inject into LC-MS/MS (Ugare *et al*., 2013) [21].

Identification of safer fungicides and days required to reduce residue below BLQ

Collected data on pesticide use history and results of each fungicide/insecticide were arrange on basis growth stages and date of pruning safe fungicide and their possible application time was identified on the basis of residue results. In case of fungicide which residue was not detected at harvest, was consider safe for period during which was actually applied. Individual pesticides detected /non detected in residue analysis were tabulated to understand the DBLQ (days required to reduce residue to below levels of quantification (BLQ). Observed DBLQ was calculated by ducting days after pruning on which last spray was given from days after pruning on residue samples was collected. Estimated DBLQ (days required after last spray to dissipate residue below level of quantifications) was calculated by pesticides dissipation data collected from, National referral Laboratory, ICAR-NRCG, Pune and data of experiment conducted by me

Dissipation data arrange in day wise analysis, load data in tablecurved 2D software, available in NRL lab, proceed data till the value 0.01mg/kg (below 10ppb) was come, which was called BLQ value of that pesticides. Residue dissipation curves can be used to estimate the time required for residues to reach levels below maximum residue limits (MRLs) (Fong *et al*. 1999) [8].

Results and Discussion

Only flusilazole and hexaconazole were detected above MRL. Flusilazole 40% EC @ 25 mL/200 L water has PHI of 60 days thereby indicating a long duration of persistence. Hence growers tend to use higher amount than recommended, so residue detection is very common. Hexaconazole recently has been banned in EU and hence its MRL has reduced to default value of 0.01 ppm instead of 0.5 ppm. Both these fungicides thus should not be preferred for use after fruit pruning.

Out of twenty one vineyards, only in six vineyards applied bioagents like Trichoderma spp., Ampelomyces spp., Bacillus spp. and Psedomonas spp. were applied for management of diseases. Biological control using Trichoderma and Bacillus subtilis are good options which can minimize the need of a large number of fungicide applications (Sawant *et al*., 2017a; Sawant *et al*., 2011) [13, 11] and can be adopted by growers.

In five vineyards bio-agents like Beauveria bassiana, Metarhizium, Verticillium lecanii were applied for managements of sucking pests. Few farmers applied 11 to 13 sprays of bio-agents for management of disease and pest. Interestingly residue detection in their cases was also less although they have applied many chemicals pesticides. Organic product Neemazal (Azadirachtin 1% EC @ 3.0 mL/L water) is recommended for management of thrips with reduced number of insecticides applications (ICAR-NRCG, Annexure-7, 2006). Biological control using Beauveria bassiana and Metarhizium are good options for management of mealy bugs with reduced number of insecticides applications (Rondot and Reinke, 2018) [11]. Verticillium lecanii WP @ 0.3% is also reported to be effective against nymphs and adults to grape mealy bug in Maharashtra (Koli, 2003) [10].

A couple of surveyed farmers who were practicing 'zero budget' farming used alternate methods like botanicals, 'Jeevaamrut' and buttermilk etc. for insect pest control instead of pesticides. They were not as concerned with blemishes on fruits as the grapes were to be dried for raisins.

Table 1: List of fungicides used whose residue were not detected

Sr. No.	Fungicides	No. of sprays	Last spray (DAP)	Observed DBLQ (days)	Recommended EU MRLs mg/kg	Recommended Indian MRLs mg/kg	PHI (days	Estimated DBLQ
	Non systemic fungicides for downy mildew							
1	Propineb 70 WP	6	85	75	1.0	-	40	125
3	Potassium salt Of phosphoric acid	3	60	96	100.0	-	-	
5	Mancozeb 75 WP	10	47	110	5.0	3.0	66	82.5
		8	60	88	-		-	-
	Systemic fungicides for downy mildew							
1	Pyraclostrobin 25% SC	1	45	110	1		34	65.5
2	Cymoxanil	4	54	105	0.3	0.10		
		3	64	90	-		-	-
3	Kresoxy methyl	2	90	92	1	-	30	68.5
	Combination fungicide for downy mildew							
1	Metalaxyl+mancozeb	2	84	125	2.0+5.0	-	66	92+125
	Non systemic fungicides for powdery mildew							
1	Tridemefon	3	45	90	-	2.0	-	
2	Dinocap	1	61	92	-	-	-	
	Systemic fungicides for powdery mildew							
1	Metrafenon	1	95	52	7	-	22	98
2	Penconazole	1	82	99	0.2	-	50	90

Table 2: List of fungicide use by growers whose residue detected

Sr. No	Fungicides	Residue detected: No. of sprays	Residue detected: Last spray (DAP)	Residue mg/kg	Obse. DBLQ	Recommended MRLs mg/kg: EU	Recommended MRLs mg/kg: India	PHI (days)	Estimated DBLQ
1	Dimethomorph	1	30	0.01	135	3	0.05	34	46.5
		3	25	0.03	94	-		-	
		3	40	0.03	104	-		-	
		2	30	-	94	-		-	-
		3	50	-	63	-		-	-
2	Mandipropamid	2	64	0.03	95	2		5	
		4	85	-	114	-		-	-
3	Iprovalicarb+pr	2	84	0.01+0	79	2+5		55	
	opineb	2	42	0.01	121	-		-	
		1	42		99	-		-	-
4	Fosetyl Al	3	35	0.06	35	100	10.0	30	
		1	90	-		-		-	
5	Pyraclostrobin+metiram	1	60	0.09+0.0	76	1+5		34	65.5
		1	42		142	-		-	-
6	Azoxystrobin	2	102	0.16	42	3		7	
7	Fluxapyroxad+pyraclostrobin	1	34	0.09	110	2+1		60	73+65.5
For powdery mildew managements									
8	Tetraconazole	2	60	0.06	97	0.5		30	34.5
		3	70	0.01	57	-		-	
		1	55	-	-	-		-	-
9	Tebuconazole	1	40	0.01	65	0.5		34	
10	Flupyram+tebuconazole	1	60	0.15	65	1.5+0.5		60	Fluyram-82
		1	40	-	47	-		-	-
11	Myclobutanil	1	80	0.01	62	1		30	
		2	70	0.1	81	-		-	-
		6	90	0.1	37	-		-	-
		1	74	-	50	-		-	-
		2	64	-	71	-		-	-
		3	93	-	70	-		-	-
12	Hexaconazole	8	85	0.14	45	0.01		60	53
		2	84	0.02	63	-		-	-
		4	85	-	63	-		-	-
		2	42	-	71	-		-	-

13	Difenoconazole	3	74	0.80	43	3		45	
		2	74	0.08	81	-		-	-
		2	65	0.23	81	-		-	-
		3	54	-	116	-		-	-
		2	70		74	-		-	-
14	Flusilazole	2	20	0.05	117	0.01		60	115
For powdery mildew managements									
	Carbendazim	1	30	0.30	112	0.3	2.0	50	
		4	70	0.03	-	-		-	-

Table 3: List of insecticides used whose residue were not detected

Sr. No.	Insecticides	Residue not detected		Recommended		Estimated DBLQ
		No. of sprays	Last spray (DAP)	EU MRLs (mg/kg)	PHI (days)	
1	Emamectin benzoate	5	110	0.05	25	67
		5	108	-	-	
2	Fipronil	5	54	-	75	
		1	90	-	-	
		3	35	-	-	

Table 4: List of insecticides used by growers whose residue were detected

Sr. No.	Insecticides	Residue detected		Residu e mg/kg	Recommended		Estimated D BLQ (Days)
		No. of sprays	Last spray (DAP)		EUMRLs (mg/kg)	PHI	
1	Lambda cyhalthrin	2	74	0.01	0.01	30	86
		4	60	0.01	-	-	-
		1	85	0.01	-	-	-
		5	70	ND	-		
		4	50	ND	-		
2	Buprofezin	5	85	0.02	1	40	94.5
		2	85	0.23	-	-	-
		1	70	ND	-		
		2	70	ND	-		
		3	95	0.39		-	-
3	Imidacloprid	4	85	0.01	-	60	
		6	45	0.03	-	-	-
		4	60	0.02	-	-	-
		3	84	ND	-	-	-
		2	50	ND	-	-	-

Systemic fungicides used for management of downy mildew

CAA fungicides

From Table 1, In case of dimethomorph, it was observed that there was a risk to use it at 30 DAP. Use mandipropamid after fruit set was risky too as one case where applied at 64 DAP twice, 0.03 mg/kg residue was detected. From Table 2. and 3 it was observed that use of iprovalicarp after 40 days was also not safe.

QoI fungicides

Azoxystrobin when applied at 102 DAP, 0.16 mg/kg residue was observed in surveyed vineyard. Pyrachlostrobin+metiram when sprayed at 42 DAP once; there was no detection of residue. Residue analysis of five samples where sprays were given before fruit set, no residue was detected. However if used at 60 DAP, 0.09 mg/kg residue was detected. It was found that Kresoxym methyl applied before 90 DAP, did not have any issue of detection of residue.

PA fungicide

Fungicide metalaxyl+mancozeb when applied twice at 84 DAP, residue was not detected, this fungicide was applied in three vineyards and residue was not detected in any of them.

Phosphoric acid groups

From Table 1, it was observed that, Fosetyl Al when applied once at 90 days after pruning, applications, there was no detection of residue. Potassium salt of phosphoric acid when used up to 60 DAP with three applications, there was no issue of residues. In four samples, where sprays were given before fruit set no residue was detected.

Other group

It was found that when Cymoxanil was applied at 64 DAP for three times, residue was not detected. It was used in 13 vineyards and around 1-4 applications were made, in all but no residue was detected if appears safe fungicides from residue point of view.

Systemic fungicides used for management of powdery mildew

From Table 2, it was found that difenoconazole when applied thrice at 54 DAP, residue was not detected. However if it was Applied twice after 65days, 0.23 mg/kg residue was observed in surveyed vineyards. Two sprays of Hexaconazole at 42 DAP did not give any residue. Nine samples where Hexaconazole sprays given before fruit set did not report any residue. However if used up to 84 DAP, chances of residue detection was more. When tetraconazole was used at 60 DAP with two applications, chances of residue detection was more. In case of tebuconazole, it was observed that there was risk to use it 40 DAP.

Myclobutanil when applied twice at 64 DAP with two applications, there was no issue of residue. Penconazole when applied once at 82 DAP residue was not detected.

SDHI group fungicide
Metrafenone when applied once at 95 DAP with one application, there was no residue. As is evident from three samples. In case of fluxapyroxad+pyraclostrobin, it was observed that there was risk if used 34 DAP. Systemic fungicide flupyram +tebuconazole, when applied at 60 DAP, there was risk for residue detection.

Benzimidazole fungicide
Carbendazim when applied once 20 at DAP, there was no detection of pesticide residue and analysis of three samples proved the same. Where sprays given before 20 days after pruning and residue were not detected. However, if it is applied after 30 days, possibility of residue detection was more.
A high number of applications chemical in a vineyard brings out the need for better awareness for management of powdery mildew using non-chemical methods. On the other hand a number of fungicides are registered for control of diseases hence growers have ample choice and generally do not use a non-label claim fungicide.

4.1.4 Insecticides:
From Table 3 and 4, it was observed that emamectin benzoate when applied five times from 110 DAP, there was no issue of residue detection. Eight samples where sprays were given before 110 DAP and residue were not detected. Fipronil when applied 54 DAP with five applications, residue was not detected. In survey, it was found that seven samples where sprays given before 54 days after pruning and there was no detection of residue. From Table 3, Lambda-cyhalothrine when four sprays 50 DAP, there was no issue of residue. After data analysis, four samples where sprays were given 50 DAP and residue was not detected. However if applied after 50 days, possibility of residue detection was more. Three samples where sprays given 50 DAP and residue were detected. Buprofezin when used twice at 64 DAP, there was no issue residue. Three samples where sprays given before 70 DAP and did not have any issue of residue. However if applied after 70 days, there was possibility of residue detection. Eight samples where sprays given before 65 days after pruning and residue were detected. Imidacloprid when applied thrice at 84 DAP, there was no issue of residue. Six samples where sprays given before 84 days with 1-4 applications and residue were not detected. However if applied after 45 days, with more No. of applications chances of residue detection were more.
As per ICAR-NRCG, Pune, annexure- 5 RMP document, only six insecticides are registered for pest management in grapes. Three chemicals are registered for control of thrips, two are registered for control of flea beetle and one for mealy bugs. These three pests can cause severe damage in grapes if left uncontrolled, hence growers take a chance by applying chemicals which have label claim in some other crop. Overall these appear to be reluctance on the part of grower to apply bio-control methods for disease and pest management. A numbers of on farm demo coupled with proper capacity building training may help in better adaptation of these methods.
Detection of pesticide residues in grapes is reported the world over. The 2015 EU report on pesticide residue states that of the 1287 samples of table grapes analysed during 2015, only 22.7% samples were residue free, 19% samples contained residues of single pesticide, while a large number of 58% samples contained multiple residues (European Food Safety Authority. 2017). Exceeding the Maximum Residue Levels does not necessarily imply a risk to health. However, it usually indicates that a pesticide has been incorrectly used. It is generally assumed that when a farmer uses a pesticide according to the label instructions and GAP, the residues in crop at harvest do normally not exceed the MRL. Farmers compliance with GAP, following the basic principle of using pesticides as little as possible and only when necessary will go a long way in producing pesticide free grapes.
Conclusion: During the flowering phase were CAA fungicides deemed safe. After fruit set, when downy mildew treatment is required, fungicide Cymoxanil is thought to be safe to use because it was not detected even when sprayed up to 60 days after pruning. When Mancozeb used in combination with potassium salt of phosphoric acid were safe up to 60 DAP.

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