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Effects of Brining and Picking Time on The Degradation of Pesticide Residue in Grapevine Leaves

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A B S T R A C T

Intensive pesticide use in vineyard resulted in residue problem on vine leaves that are used as food. This study was aimed at development of a proper chemical control program to reduce the pesticide residue problem on vine (cv. Narince) leaves in vineyards during the growing period. The residues of some fungicides were determined according to spraying time in the fresh (unprocessed) and preserved (brined) leaves. Additionally, the effects of preservation process on degradation of the fungucides residues were investigated. In this study three fungicides (*Azoxystrobin*, *Triadimenol*, *Hexaconazole*) were applied alternately for both powdery mildew and "*Colomerus vitis"* management, and two fungucides (Copper oxychloride**,** Metalaxyl + Mancozeb) for downy mildew control. Additionaly vine leaves were harvested at two different times: (i) before the half-life of the pesticides were reached and (ii) after the half-life of the pesticides have elapsed. Two different methods were applied to preserve the vine leaves. In first treatment, leaf samples were boiled in hot (98±2°C) tap water, then leaves were placed into jars, then filled with brine containing 8.0% salt + 0.25% lactic acid. In second treatment, vine leaves were placed into jars, then filled with tap water and brine containing 8.0% salt+0.25% lactic acid. The residue levels of the fungicides were determined on leaves. Detectable copper and the other fungucide residues are compared according to Turkish Food Codex. Preserving applications were decreased fungicide and copper residue levels and hot water brining was decreased the levels of fungicide residues between 75.2% and 99.2%, according to the applications. As a result, systemic fungicides should not be used in vineyards in where pickled vine leaves are produced. It is proposed that better to use contact fungicides instead of systemic one and also viticulturists should be careful using the effective contact fungicides.

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Introduction

Grape is used for different purpose while grapevine leaves have been traditionally used as a food in both fresh and brined forms in Turkey. The leaves are used to make stuffed grapevine leaves after gathering as fresh leaves or canned. Grape leaves contain several vitamins and minerals (El Nehir, et al., 1997; Gülcü and Demirci, 2011). Fresh grapevine leaves tend to decompose in a very short time during storage. Therefore, people generally prepare different kinds of brines to preserve the leaves for future use (Koşar et al., 2007). The grape variety affects the quality of pickled grape leaves. Thick and hairy leaves with lobes are not preferred by consumers. The leaves of 'Narince' and 'Sultani Cekirdeksiz' cultivars are widely used for pickled grapevine leaves products in Turkey (Gökturk et al., 1997). Harvest of brined grapevine leaves usually start when the summer shoot reach 50 cm long (after flowering) and continue till beginning of fruit maturity. According to the producer and region brined vine leaves are harvested 2 or 7 times a year.

The current model of agriculture is designed to maximize profit by increasing production yields and quality of agricultural products, while reducing costs for both producers and consumers. To reach this goal we should develop effective management programs for pests and diseases. Pesticides still play important role in a successful pest management program, despite their disadvantages such as toxicity to non-target organisms, environmental pollution and residue (Kaushik et al., 2009).

Intensive use of chemical pesticides for control of grape pests in commercial grape production is one of the main management practices. During the harvesting period of vine leaves, different contact and systemic fungicides are applied to control *Phomopsis viticola* Sacc., *Plasmopara viticola* "B. et. C. and *Erysiphe necator* "Schwein" Burr. in vineyards. Both brined grape leaves and grape are produced in the same vineyards. This kind of production system resulted in reduced grape quality and pesticides residues on leaves and fruits. Tokat province is one of the main grape production areas of the Turkey and pesticides residues on grape leaves and fruits are the most important problem of vineyards of Manisa and Tokat provinces (Cangi et al., 2005; Yanar et al., 2015).

The pesticide residues, left to variable extent in the food materials after harvesting, are beyond the control of consumer and have deleterious effect on human health. There are several studies on pesticides residues on table grapes, raisin (Kaya et al., 2000; Pire, 2001; Turgut et al., 2011) and wine (Şen, 2005; Cus et al., 2010; Regueiro et al., 2015), on the other hand number of studies on pesticide residues on brined grapevine leaves are limited (Shokr et al., 2006; Ertürk, 2009; Cangi et al 2014; Şensoy et al., 2017; Bakırcı et al., 2019; Kuşaksız and Çimer, 2019; Tutku and Tuna, 2019). In other study, indicate that fenarimol and flusilazole were determined more persistent in grape leaves than fruits of grape (Shokr et al., 2006).

Ahmad Sama'neh (2003) investigated detection of Chlorpyrifos and penconazole in grape leaves by GSMS; Nasr et al. (2003) studied boiling and some environmental factors on residues behaviour of penconazole fungicide on grapevine leaves; Erturk (2009) determined fungicide residues in fresh and pickled grape leaves (cv. Yapincak) grown in Tekirdag province; Yanar et al. (2015) detected pesticide residues in fresh and pickled vine leaves in Tokat province. In a study conducted by Yanar et al. (2015), the residues of Triadimenol, Azoxystrobin and Metalaxyl were detected in most of brined grapevine leaf samples were over the MRL. Bakırcı et al. (2019), in their study in the province of Manisa 232 vine leaves investigated the pesticide residues. They found eighty-five (36.6%) pesticide residues in 52 samples (22.4%). They found that metalaxyl was the most active substance in the vine leaves, and that the active substance on the TGK MRL values was azoxystrobin.

Every pesticide needs time to reach half-life before harvesting a crop. If the conditions of good agricultural practice (GAP) such as applicable dose of the pesticide and the time interval between application and harvesting the crop are not met, harvested crops may contain unacceptable levels of pesticide residues (Gonz´alez-Rodr´iguez et al., 2011). Some studies have shown that certain types of postharvest treatments or household preparations may help to reduce pesticide residues (Ramesh et al., 1999; Zabik et al., 2000; Krol et al., 2000; Dhiman et al., 2006; Aktar et al., 2010). The washing with water or soaking in solutions of salt and some chemicals (chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, hydroxy peracetic acid, and detergents) are reported to be highly effective in reducing the level of pesticides. Various thermal processing treatments like pasteurization, blanching, boiling, cooking, steaming, canning, scrambling etc. have been found valuable in degradation of various pesticides depending upon the type of pesticide and length of treatment (Shokr et al., 2006; Bajwa and Sandhu, 2014; Regueiro et al.,2015; Kuşaksız and Çimer, 2019). Also fermentation is often part of food-processing operations, which can reduce the pesticide residue on/in crops. (Pardez-L´opez et al., 1991). In a study conducted by Lu et al. (2013) reported that the pickling process had reduced the amount of pesticide residual in pickled cabbage.

In a vineyard pesticide application program, recommended by The Republic of Turkey Ministry of Agriculture and Forestry, was determined for grape not for grapevine leaves. So there is no data about the time should be elapse between spray and harvest for brined grapevine leaves. So that appropriate pesticide application program should be established for the vineyards which used for both grape and leaves productions.

The study was conducted to develop pesticide application program suitable for both grape and leaf production and to determine the effect of fermentation process on degradation of fungicide residue of grapevine leaves.

Materials and methods

Material

The study was conducted in 2011 growing season at a ten-year old *Vitis vinifera* L. cultivar 'Narince' grafted onto 1103 P in Tokat provinceof Turkey. The row and vine spacings were 3.0 and 1.75 m, respectively. Vines were trained as bilateral cordon onto single-curtain (wire) trellis system.

The brined leaves of 'Narince' grape cultivar are shown in Figure 1. Kara (1990), some of ampelographic characters determinate for 'Narince' grape cultivar. Colour of young leaf (4-6. leaf), Bronze speckled green; shape of mature leaf, pentagonal; mature leaf area, 232.67 cm^2; colour of mature leaf, dark green (PL-XXIX-421); bunch weight, 227.65 g; berry weight, 3.34 g.

Figure 1 Upside and underside view of the brined grapevine leaf of Narince cultivar

Pesticides Applications and Vine Leaves Pickling

The pesticides doses and the time should be elapse were determined according to the manufacturer's directions. Pesticides applications program and time interval between applications and leaves sampling were given in Table 1. All the vines were sprayed with micronized sulphur and metiram when annual twins reach 20-25 cm long to control powdery and downy mildews. Experimental design was randomized block design with four replication and each replication consist of 10 grapevines.

Grape leaves were taken at the half of time and at the end of the time which should elapse between application and harvest. Healthy undamaged leaves with normal appearance were harvested from the third to fifth leaf stage (up to two thirds of mature leaves) from the apex.

Fresh leaves (unprocessed) samples (500 g per treatment) were taken and stored in deep freeze at -18°C for pesticide residue analysis (Anonymous, 2002).

Canning Process

The leaves were cleaned and classified according to their size suitability for processing and their petioles were shortened to 2- cm, then leaves were placed into sealing glass jars (1000 cc). Two different methods were applied to preserve the vine leaves. First method, hot water brine, leaf samples were boiled in hot (98±2°C) tap water for 15 minutes, then filled with brine water containing 8% salt + 0.25% lactic acid. Second method, cold water brine, vine leaves were placed into jars, then filled with tap water (22±2°C) and brine containing 8% salt + 0.25% lactic acid. The leave samples were stored to fermentation for 3 months in ambient temperature (22±2°C).

Residue Analyses

Then residue analyses were made separately fresh and preserved vine leaves. Fresh and brined vine leaves residue analysis was performed in Manisa Province Control Laboratory. Extraction procedure was performed according to the methods of Lehotay (2005). Residue analysis were done by use of Liquid chromatography–tandem mass spectrometry (LC–MS/MS) LC-MS/MS Waters model ACQUITY UPLC-TQD(MS/MS) system and Perkin Elmer model Clarus 500 MS system.

Residue limits adopted for vine leaves were given Turkish Food Codex (TFC) according to (Anonymous 2016) in Table 2.

Table 1 Spray program and grapevine leaves sampling dates after spray

Application code	Fungicide applied (*)		Time elapse between spray and harvest
	For powdery mildew	For downy mildew	
A	Azoxystrobin	Copper oxide	11. and 21th day
B	Azoxystrobin	Metalaxyl + Mancozeb	8. and 21th day
C	Triadimenol	Copper oxycloride	11. and 21th day
D	Triadimenol	Metalaxyl + Mancozeb	8. and 21th day
E	Hexaconazole	Copper oxycloride	8. and 21th day
F	Hexaconazole	Metalaxyl + Mancozeb	8. and 14th day
G	Control		11th day

*Fungicide applications were made when the berries reach the size of lentil.

Table 2 Maximum residue limit (MRL) values of some pesticide on edible grapevine leaves according to the Turkish food codex (TFC) (Anonymous, 2016).

Active ingredient	MRL values (ppm) for vine leaves
Azoxystrobin	0.01*
Hexaconazole	0.01*
Metalaxyl	0.05*
Metiram	0.05
Triadimefon ve triadimenol	0.1*
Sulfur	50.0
Copper	20.0+

*The lowest analytically detectable limit (LOD)

Results and Discussion

In Turkey, brined grapevine leaves become an important source of income for grape producers. But in a vineyard where grape is produced as a main crop Copper, sulphur and other pesticides residues occurring most frequently. In this study, level of pesticides residues on vine leaves were determined under controled pesticides application program.

In our study, copper (Copper oxycloride, 50%), metalaxyl, mancozeb, azoxystrobin and triadimenol were applied on grape vine and the leaves samples were collected at different times after applications. Based on the residue analysis results, copper oxycloride and the other fungicides residues in grapevine leaves harvested before half-life of the fungicide were higher than the limits of TFC. Sulphur and metiram residues were not detected on fresh and brined leaves in any samples. The fungicides residues were decreased by extending the harvesting time after spraying on fresh leaves in all treatments (Table 3). There were no pesticides residues in vine fresh leaves collected from control treatments, however, the residues of azoxystrobin, triadimenol, hexaconazole and copper in the fresh leaves from treatments have been above the MRL at all picking periods after applications. On the other hand, amounts of metalaxyl at leaves collected from treatment B 8 days after application was higher (0.05 ppm) then the limits of TFC, but there was no metalaxyl residue after half-life of the fungicide over. If we consider that the half-life of metalaxyl was 14 days in vine, this was the expected results. In treatment D, triadimenol and metalaxyl was applied in a mixture and residue level of metalaxyl was higher then the limits 8 days after metalaxyl application but 21 day after application metalaxyl residue was not determined (Table 3).

In treatment E amounts of Copper oxychloride were higher than MRL at 8 and 21 days after applications. However, metalaxyl residue in vine leaves in treatment F was above the limits 8 days after application, no samples contained metalaxyl at half-life of the fungicide (14 days after application). Mancozeb residue was not detected in fresh leaves in treatment (Table 3). This is evidence that the fungicide residues decrease by increasing time after spraying (Table 3). Similarly, the chlorpyrifos and penconazole residues were decreased by increasing time after spraying on grape flesh, cortex and leaves (Ahmad Samaneh, 2004).

The level of pesticide residues is affected by cold and hot water brining applications and by increasing time after spraying on brined leaves in all treatments. But, residues of azoxystrobin and hexaconazole in the brined leaves have been above the MRL after spraying in all treatments. Residue of triadimenol on brined leaves was detected under the MRL. Metalaxyl residues were varied according to treatments. Hot water brining was the most effective method for degradation of pesticide residues in grapevine leaves (Table 4, 5, 6).

Table 3 Copper and other fungicide residues in fresh unprocessed grapevine leaves

TC	HTS (day)	Amount of fungicide residues determined in fresh grape leaves (ppm)					
		Azoxystrobin	Triadimenol	Hexaconazole	Metalaxyl	Mancozeb	Copper
A	11	2.73					250.5
	21	0.532					96.54
B	8	3.789			0.896	ND	
	21	1.654			ND	ND	
C	11		0.203				42.57
	21		0.033				14.08
D	8		0.505		0.689	ND	
	21		0.310		ND	ND	
E	8			1.658			168.6
	21			0.950			16.69
F	8			1.896	0.570	ND	
	14			1.089	ND	ND	
G	11	ND	ND	ND	ND	ND	ND

TC: Treatment codes, HTS: Harvesting time after spraying, ND: no detected

Table 4 Copper and other fungicides residues detected on cold water brined grapevine leaves

TC	HTS (day)	Amount of fungicides residues (ppm)				
		Azoxystrobin	Triadimenol	Hexaconazole	Metalaxyl	Copper
A	11	1.72				173.1
	21	0.39				83.93
B	8	1.96			0.762	
	21	0.10			0.015	
C	11		0.190			25.50
	21		0.140			13.7
D	8		0.120		0.145	
	21		0.099		0.010	
E	8			0.052		150.7
	21			0.040		12.67
F	8			0.046	0.098	
	14			0.066	0.045	
G	11	ND	ND	ND	ND	ND

TC: Treatment codes, HTS: Harvesting time after spraying, ND: no detected

Table 5 Copper and other fungicides residues detected on hot water brined grapevine leaves

TC	HTS (day)	Amount of fungicides residues (ppm)				
		Azoxystrobin	Triadimenol	Hexaconazole	Metalaxyl	Copper
A	11	0.091				143.2
	21	0.015				78.83
B	8	0.052			0.050	
	21	0.013			0.025	
C	11		0.012			23.54
	21		0.050			11.9
D	8		0.090		0.088	
	21		0.011		0.015	
E	8			0.410		103.7
	21			0.039		10.02
F	8			0.389	0.053	
	14			0.059	0.038	
G	11	ND	ND	ND	ND	ND

TC: Treatment codes, HTS: Harvesting time after spraying, ND: no detected

Maximum reduction rates of azoxystrobin residues in grapevine leaves (96.7-992%) was observed by hot water brining, however the reduction ratios were lower in cold water application. The residues of triadimenol were reduced by 822-965% by hot water brining in grapevine leaves. The reduction of hexaconazole residue was found in approximately the same ratios in both brine applications. The residues of metalaxyl were reduced by 15.19-94.4% with hot brining water application. The degradation ratios of copper residues with hot brining application were found between 15.6-44.8%, however the reduction ratios were lower in cold water application (Table 6). Similarly in a previous study, washing with water or soaking in solutions of salt and some chemicals e.g. chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, hydroxy peracetic acid, iprodione and detergents are reported to be highly effective in reducing the level of pesticides (Bajwa and Sandhu, 2014).

There were many studies conducted to determine the pesticides residues in table, wine grapes and wine in the world (Kaya et al., 2000; Pire, 2001; Cus et al., 2010; Turgut et al., 2011). But, studies on pesticides residues in grapevine leaves are limited. In the study conducted in Slovakia vineyards to determine the netalaxyl residues which were applied at a rate of 0.25 kg/ha and 2.5 kg/ha there were no metalaxyl residues one month after applications (Vasilieva et al., 1991).

Based on the present study results, cold and hot water brining may result in reduction of pesticides residues in vine leaves. But it seems that this reduction will not be under the MRL levels for all active ingredients.

Similarly in a previous study brining reduced the residues of triadimenol, triadimefon and folpet on Yapıncak grape cultivar in Tekirdag-Turkey, and the residues of triadimenol, triadimefon and folpet after brining were 7.348 ppm, 0.137 and 722 ppm, respectively. On the other hand residue levels of triadimenol, triadimefon and folpet in un-brined control were 2980 ppm, 137 ppm, and 1722 ppm respectively (Ertürk, 2009).

In another research, boiling process was reported that very effective in eliminating fenarimol residues on and in grape leaves than flusilazole residues. Whereas the reduction of fenarimol and flusilazole residues in the leaves due to boiling process were 47.06% and 24.76% for leaves picked one day after spraying, with residues decreased from 0.340 and 0.210 ppm in the fresh leaves to 0.180 and 0.158 ppm in the boiled leaves (Shokr et al., 2006).

Cangi et al. (2014), reported that brining application were generally decreased fungicides residue levels, but not all the time brining was not affected the carbendazim residue on brined grapevine leaves. Also it was reported that hot water brining method resulted in higher residue reduction (91% reduction compared to fresh leaves) than the cold water brining method (21% reduction compared to fresh leaves). In the similar study, it was determined that the pesticide residue values of grape leaves decreased in the cold brine by 69-73 % and in the hot brine by 73-91% (Kuşaksız and Çimer, 2019). Results of some studies shown that pesticides residues might be reduced when fresh vine leaves were washed, boiled and then brined (Nasr et al., 2003; Shokr et al., 2006; Ertürk, 2009; Cangi et al., 2014; Gülcü and Demirci, 2014; Kuşaksız and Çimer, 2019), however washing did not reduce penconazole residues in vine leaves (Batta et al., 2005). Kang and Lee (2005) reported that brining had little impact on pesticide levels in cabbage apart from those of diazinon and dichlorvos, decreased by about 20%.

Residues of pesticides in food are influenced by processing such as fermentation. Reductions of pesticide levels during fermentation could mainly be due to chemical or biological degradation (Aislabie and Lloyd-Jones, 1995; Azizi, 2011; Lu et al., 2013), rather than the absorption onto the microbial cell walls (Ruediger et al., 2005).

In order to decrease the risk of taking high concentration of both pesticides during eating fresh leaves or brined leaves, it is important to respect to the time needed for degradation of these pesticides due to the action of internal metabolic processes depending on the time passed after spraying.

Conclusions

In general, systemic pesticides residues are found more frequently than the contact pesticides. However there is no record on the package of brined vine leaves when and where the leaves are collected. Therefore how soon the pesticides residues in vine leaves may fall below MRL levels. Present study revealed that how long the systemic pesticides may stay in vine leaves when the leaves harvested at different time after the pesticide application.

Table 6 The degradation rates of copper and the other fungicides residues with cold and hot brine applications (%)

TC	HTS	Azoxystrobin		Triadimenol		Hexaconazole		Metalaxyl		Copper	
	(day)	CWB	HWB	CWB	HWB	CWB	HWB	CWB	HWB	CWB	HWB
A	11	36.9	96.7							30.8	42.9
	21	26.7	97.2							13.0	18.3
B	8	48.3	98.7					15.17	94.4		
	21	93.9	99.2					-	-		
C	11			6.4	94.1					40.14	44.8
	21			-	-					2.8	15.6
D	8			76.2	82.2			78.9	87.2		
	21			68.1	96.5			-	-		
E	8					66.9	75.2			10.6	38.5
	21					95.8	95.9			23.9	40.1
F	8					79.4	97.6	82.8	85.4		
	14					93.9	94.6	-	-		

TC: Treatment codes, HTS: Harvesting time after spraying, CWB: Cold Water Brine, HWB: Hot Water Brine (%)

The growers whom grow grapevine for brined vine leaves production first of all they should prefer leaves production to grape production. Grapevine leaf producing manufacturers shouldn't use systemic pesticides during leaves harvest period. We recommended the use of contact pesticide instead of systemic one.

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