10.7251/AGSY1303553K

RESIDUES FOLPET IN GRAPE ŽILAVKA AND BLATINA VARIETIES FROM PLANTATION CULTIVATION AND IN THE SMALL VINEYARD

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Summary

The application of pesticides is a necessary measure in the cultivation of grapevine. Fungicides, in comparison to other pesticides, are the most often used in vineyards due to the sensitivity of grapevine on the most common diseases caused by pathogenic fungi (*Plasmopara viticola, Uncinula necator* and *Botrytis cinerea*), which reduce the yield and quality of grapes. Frequent use and misuse regardless respecting of preharvest period, causes acumulation of fungicide active substances in grapes, and then their transfer into the wine. In this paper the results of the residues of folpet in two most frequently grown varieties of wine grape (Žilavka and Blatina) from plantation cultivation and in the small vineyard are presented. The device GC/MS Agilent 7890A/5975C and analysis method UNI EN 15662:2009 QuEChERS was used for determination of residues of folpet. Determined concentrations of residues of folpet in grapes of Žilavka and Blatina grown in 2011 in two plantations and in the small family vineyard were below the MRL value (Maximum Residue Levels) determined for folpet with Regulation (EC) No. 396/2005, which for wine grapes is 5 mg / kg as well with Regulations on the quantities of pesticides and the other toxic substances, hormones, antibiotics and mycotoxins that may be present in food ("Official gazette of SFRJ", No. 59/83 and 79/87) that for folpet is 2 mg/kg.

Key words: grapes, folpet, MRL, Žilavaka, Blatina

Introduction

Use of fungicides has special significance in successful control of the most often grapevine diseases (*Plasmopara viticola, Uncinula necator* and *Botrytis cinerea).* The most commonly used fungicides for grapevine protection are procymidone, cyprodinil, fludioxonil, myclobutanil, iprodione, folpet, vinclozolin (Flamini and Ponighel, 2006). Excessive use of fungicides indicates necessity for control of their residues in grapevine and wine. In Europe, grapevine is food that is the most contaminated with pesticide residues. The report published by Enviromental Working Group (EWG) for 2010 indicate that grapevine is in the group of 12 food items with the most often reported pesticide residues. Residues of these compounds are one of the most important contamination sources in production of fruits and vegetables, and may present hazard for health of consumers and environment. The studies published in 2007 shows that grapevine is among 24 different food items analysed within annual food monitoring EU programmes in the period 2001-2005 and it holds fourth place considering fungicides residues quantity after pears, oranges and strawberries[2]. According to literature related to problems of fungicides residues in grapevine during harvest and during wine production, folpet residues are the ones that the most commonly may be found. Results of the study Baša - Česnik et al. (2008) regarding monitoring of pesticide residues in 47 grapevine samples showed the most often presence of folpet (97,9%), then cyprodinil (51,1%), dithiocarbamates (44,7%), hlorotalonil (23,4%), chlorpyrifos (19,1%) and pyrimethanil (14,9%).

[2] Internet, Annual EU-wide Pesticide Residues Monitoring Report – 2001-2005, European Commission (2007)

Soleas and Gilbderg (2000) analysed 26 pesticides in 1827 samples of must prepared for fermentation, and in 1537 vine samples, from nine world regions (Australia, Canada, Central Europe, France, Italy, South Afrika, Spain and USA), folpet (14,5%) and captan (13,9%) were mostly detected, followed by carbaryl (7,9%), malathion (3,5%) and dimethoate (2,5%). Folpet residues in squeezed white grape of „Godello“ variety, were 0,34 mg/kg and they were below the limit of vine determination that is under EU MRL (5 mg/kg) (Gonzalez-Rodriguez, 2011). During vinification process folpet is quickly degraded by photo-lytic and hydro-lytic reactions (Cabras et al., 1997 – according to Čuš et al., 2010b). Results of syudy presented by Mlikota (1993) showed that alcoholic fermentation of grapevine must is influenced by folpet residues, while vinclozolin, procymidone and dihlofluanid do not effect it at all. All fungicides had good reaction on grey mold and residues were below. Mlikota et al. (1996) implies that fungicides of older generation, such as folpet and captan, have negative influence on fermentative activity of wine yeast. According results presented by Čuš et al. (2007), who monitored concentration and dynamics of active substance degradation in grapevine produced by integrated production principles, all foplet concentrations in white grapevine variety were below MRL. For red grapevine variety, folpet concentration in the amount of 0,08 mg/kg was detected in the phase of grape pulp, while in all later samples concentration was not over the determination limit. Same authors (2010) were investigating during 2007, the content of pesticide residues in Slovenia in two white (Malvasia and Pinot Gris) and two red varieties (Blaufränkisch and Refosco) of grape during grape ripening and through vinification process. Residue concentrations were decreasing in three varieties (Malvasia - 1,99 > 0,58 > 0,10 mg/kg; Pinot Gris - 2,77 > 1,23 > 0,68 mg/kg and Refosco – 3,39 > 1,27 > 0,41 mg/kg). Folpet was determined in must of white variety Pinot Gris, through vinification process in the concentration of 0,04 mg/kg, while it was not determined later on. Residues of folpet in Refosko variety were determined in squeezed grape and they were in the amount of 0,20 mg/kg. Results of Rial Otero et al. (2004) who analysed samples of grape on residues of 15 funcides, showed presence of folpet in 2 samples in concentrations of 0,1 and 0,4 mg/kg which is below national MRL (Spain). Vinification process decreases pesticide level and their content in vines is significantly lower in vines comparing to fresh grapes. Consequently, methods for detection of residues pesticide have to be very efficient and sensitive (Wong, Halverson, 1999). Unterweger et al. (1997) described GC/MS method for folpet residues detection in grape juice, must and vine.

The aim of this paper was detection of presence and concentration of a residue from active substance folpet in grape varieties Žilavka and Blatina which are grown in plantation vineyard and in the small family vineyards in the wider area of the city Mostar.

Material and methods

The research was conducted in 2011, in vineyards within the area of the city of Mostar, at four locations. Three of them (Humčine, Mukoša and Žitomislic) are plantations, while location Hodbina is small family vineyard (Table 1). In this study we are investigated the presence of folpet residues in Žilavka and Blatina, the most common grape varieties in Herzegovina.

Table 1. Basic data on vineyards whose grape was sampled

Location	Vineyard surface	Vineyard age	Grape variety
1. Humčine	2 ha	9 years	Žilavka and Blatina
2a. Mukoša	13 ha	5 years	Žilavka
2b. Žitomislic	30 ha	7 years	Blatina
3. Hodbina	0,4 ha	6 years	Žilavka and Blatina

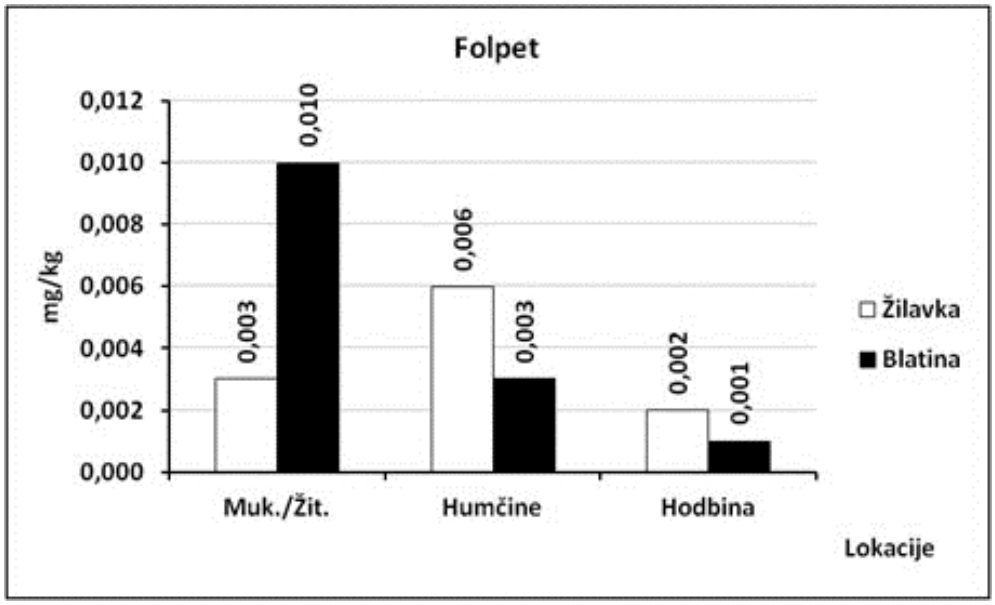
Sampling grape was done during the ripening period. The average sample was taken from five grapevines with different heights and foreign vines. The average weight of every sample was around 2 kg, with 5 bunches at least. The samples were placed into plastic bags and stored in portable refrigerator before delivering to the laboratory. The content of sugar in grape was determined on the spot, using hand refractometer 32 ATC. Grape samples which were chosen for determination of total acidity, were separate packed in plastic bags and transported to the laboratories of Federal Institute for agriculture in Sarajevo for further analyses. Determination of residues was also carried out in the same laboratory. Analytic standard of folpet was purchased at Mikro+Polo company. Characteristics of used standard were: Folpet – Sigma Aldrich, 250 mg; Batch: SZBA 0,25×V; Cleanliness: 99,7%. In term to determine concentration of folpet residue, GC/MS Agilent 7890A/5975C device was used as well UNI EN 15662:2009 QuEChERS analyses method. Basic characteristics of method which were used for this research are: Column: GC DB SMS; Port Number 190915-433; Length (m) 30 Diam (mm) 0,250 Film (μm) 0,25; Injector: Split-Splitless Inlet 280^{0}C; Colon pressure: 21,719 psi; Total Flow: 19,992 ml/min; Cromatography period (Run Time): 38,517min. The protection program grape based on folpet at the examined locations, is presented in the Table 2.

Table 2. Folpet program protection for grape varieties Žilavka and Blatina at surveyed localities

LOCATION									
No.:	**Hodbina**			**Humčine**			**Mukoša/Žitomislic**		
	Preparation	Aactive substance	Application (kg/ha)	Preparation	Aactive substance	Application (kg/ha)	Preparation	Aactive substance	Application (kg/ha)
1.	FANTIC F	Benalaksil 4% + folpet 48%	2,5	FANTIC F (2×)	Benalaksil 4% + folpet 48%	4,9	PERGADO F	Mandipropamid 5% +40% folpet	2
2.				FOLPAN	Folpet 50%	2,5	FORUM STAR	Dimetomorf 11,3% + folpet 60%	2
3.							MIKAL PREMIUM F	Fosetil 50% + 25% folpet + 4,1% iprovalikarb	3

Results and discussion

Results of our survey regarding contents of foplet residues in grape varieties Žilavka and Blatina are preseneted in Graph. 1.



Graph 1. Contents of foplet residues (mg/kg) in grape varieties Žilavka and Blatina in locations Mukoša/Žitomislic, Humčine and Hodbina in 2011

Contents of folpet residues in grape samples (Žilavka and Blatina) from locations Humčine and Hodbina were ranged from 0,001 to 0,006 mg/kg. Samples of Blatina variety from location Žitomislic contained the highest residues of folpet (0,010 mg/kg). Samples of Žilavka variety from locations Humčine and Hodbina contained double higher concentration of folpet residues in comparison with Blatina variety. Taking into account locations/vineyards, the highest concentration of folpet residues was detected in location Mukoša/Žitomislic (0,0065 mg/kg), while the lowest were detected at the location in Hodbina (0,0015 mg/kg). Grape variety Blatina contained averagely it contained more folpet residues (0,0047 mg/kg), comparing to the grapes of Žilavka variety (0,0037 mg/kg).

Thr protection program grape in the vineyard plantation Žitomislic was the same as the program was conducted in the vineyard plantation Mukoša. Regarding the same group of agronomists together with same applied agro-technical measures, in order to fulfil all demands necessary for correct analyses of variance, vineyards from Mukoša and Žitomislic were treated as one vineyard/ location. Results of analyses of variance of folpet residues are present in Table 3.

Table 3. Analyses of variance of content of folpet residues (mg/kg) in grape varieties Žilavka and Blatina in 2011 in locations Mukoša/Žitomislic, Humčine and Hodbina

Analyses of variance					
Variability source	Deviance	Degrees of freedom	Variance	F_{exp}	$F_{tabl.0,05}$
Cultivar	0,00001	1	0,00001	0,49	4,41
Location	0,00009	2	0,00005	2,37	3,55
Interaction	0,00012	2	0,00006	3,26	3,55
Error	0,00034	18	0,00002		
Total	0,00057	23			

Results of analyses of variance showed that location of vineyar (Mukoša/Žitomislic, Humčine and Hodbina) and grape variety (Žilavka and Blatina) didn't have statisticaly signigicant influence on concentration of folpet residues.

Conclusion

According results of this survey, it can be concluded that determined concentrations of folpet residues in grape varieties Žilavka and Blatina were far below MRL (Maximum Residue Levels) determined for folpet through the Regulation (EC) No 396/2005[3] and Regulations on quantities of pesticides and the other hazardous substances, hormons, antibiotics, and mycotoksins that may be present in food („Official Gazzette SFRJ“, No.: 59/83 and 79/87). This regulation as MRL for folpet in grapevine determines 5 mg/kg, while 2 mg/kg is allowed in the accordance to the SFRJ regulations. Additionally, determined concentrations of folpet residues are significantly below the values that are mentioned in numerous publication that dealt with this issue.

Study indicated appropriate application of folpet, without consequences to the residues in grapes and its processed products. However, constant work with grape producers, adoption and implementation of quality regulations and with development of analytic- research infrastructure, is necessary to insure constant supervision over fungicide residues and the other means used for protection of grapes.

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