J. Agric. Sci. Mansoura Univ., 34 (5): 5349 - 5355, 2009

PERSISTENCE OF SOME PESTICIDES ON AND IN GRAPE AND GRAPE LEAVES

Salama, E. Y.

Central Lab. of Residue Analysis of Pesticides and Heavy Metals in Food Agriculture Research Centre. Ministry of agriculture

ABSTRACT

Ebshoai and Sennoris are two cities were selected in Fayoum governorate to evaluate four pesticides {azoxystrubin, l-cyhalothrin, fenhexamid and cyprodinil } for persistence and preharvest intervals. Grape and their leaves were sprayed with three fungicides and one pesticide in two mentioned areas, a represented samples were collected from initial time passing through 1, 4,7,10,13,16 and 21 days. A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method were used for the extraction and clean-up and the pesticide residues were determined by injection on LC-MS/MS, The method were validated, the results showed that the recoveries were more than 80% and the coefficient of variation were less than 12%.

Results of analysis revealed that most of the PHI for the four compounds were not more than 21 days, Azoxystrubin showed a decay from 0.53 and 0.77mg/kg to 0.15 and 0.45mg/kg in grape and grape leaf after 21 days. l-cyhalothrin residue was decayed from 3.41 to 0.19mg/kg and from 1.76 to 0.33 mg/kg for grape and grape leaf consequently with a steep decline in the first day. Another disintegration was found in fenhexamid residue from 2.86 to 0.46mg/kg and from 1.85 to not detected after the studied time. Cyprodinil has shown a decay in its residues from 2.36 to 0.58mg/kg and from o.15 to not detected for grape and its leaf after 21 days from spraying.

Comparing all residue levels with the legislated Maximum Residue Limits by Codex Alemintarius Committee (CAC) and European committee . All grape are acceptable since the MRL's of azoxystrubin, cyprodinil and fenhexamid established for table grape are 2, 3 and 5 mg/kg consequently, whereas in case of grape leaf for the last three fungicides were 0.05 for each of them. Level of cprodinil and fenhexamid detected on grape and grape leaf are within the acceptable limits of (CAC), after on weak of application however, azoxystrubin on grape leaf exceeded the EU MRL that could be reduced after carried out processing such as washing, boiling before consumption.

INTRODUCTION

Pesticides are used in crop protection and their residues on the crops at pre-harvest may be transferred into the processed products Franck *et al* (2005). Grape is susceptible to many diseases such as Powdery and downy mildew, Ripe rot, Bitter rot, Phomopsis and Botrytis, which cause up to 30% loss in production of grape in many grape growing regions around the world Ellis *et al.* (2004); Erincik *et al.* (2001). In the recent years, attention has focused on food safety. There has been an increasing interest in novel, broad spectrum fungicides that has been designated as reduced risk pesticides to humans, non target organisms and environmental resources which belong to the new generation of fungicides used for fungal disease control in different agricultural crops. Among which fenhexamid act as a foliar fungicide with protecting action not translocated. and used for control of *Botrylis cinerea, Monillia* and related pathogens in grapes, berries stone fruit, citrus and

vegetables Nita *et al.* (2007); Wedge *et al.*(2007) and Schilder (2000). Cyprodinil is a systemic product with uptake into plants after foliar application and transport throughout the tissue and in the xylem. Azoxystrubin is a fungicide with a protectant, curative, eradicant, translaminar an systemic properties. Inhibits spore germination and mycelial growth. Cyhalothrin is a non-systemic insecticide.

The four pesticides have been tested for their persistence under local environmental conditions and the detected amount of pesticide residues has been compare to the legislative maximum residue limits.

Experimental

Reagents and chemicals

(a) Acetonitrile (MeCN), methanol (MeOH), and water.-The organic solvents we sufficient quality for pesticide residue analysis and were obtained from Labscan (Dublin, Ireland). Deionized water was used for preparing the LC mobile phase and as a reagent blank.

(b) Magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl).-Reagent grade anhydrous $MgSO_4$ in powder form, and ACS-grade NaCl were obtained from Merck (Darmstadt, Germany). The $MgSO_4$ was baked for 5 h at 500ºC in a muffle furnace to remove phthalates.

(c) Organic acids.-Glacial acetic acid (HAc) and formic acid (both from Merck) were used to improve stability of base-sensitive pesticides in the final extracts and as an acid modifier of the LC mobile phase, respectively.

(d) Pesticide standards.

Pesticide reference standards were obtained from Dr. Ehrenstorfer (Augsburg; Germany) and Sigma-Aldrich/Fluka/Riedel-de-Haen (Zwijndrecht, The Netherlands).

(e) Buffer-salt-mixture for Second Extraction and Partitioning: weigh 4g±0.2g of magnesium sulfate anhydrous, 1g±0.05 g of sodium chloride, 1g±0.05 g of trisodium citrate dehydrate and 0.5g±0.03g of disodium hydrogencitrate sesquihydrate into 25 ml glass tube.

Instruments and apparatus

(a) Liquid chromatography/tandem mass spectrometry (LC/MS-MS) instrument provided with an Agilent 1200 Series HPLC instrument coupled to an API 4000 Qtrap MS/MS from Applied Biosystems with electrospray ionisation (ESI) interface.

(b) Centrifuge.-1) For the 50 mL centrifuge tubes, a Sigma (Ostenrode am Harz, Germany) E3-1 centrifuge was utilized.

(c) Analytical balance.-A top-loading balance with digital display was used to weigh the chopped samples and powder reagents.

(d) Vials and vessels.-For both the extraction and dispersive-SPE cleanup steps, 50 mL fluorinated ethylene propylene (FEP) centrifuge tubes with ethylene-tetrafluoroethylene (ETFE) screw closures (Nalgene, Rochester, NY; USA) were employed. Sealable 15- mL glass screw-cap vials were used to contain the 6 g anh. $MgSO_4$ + 1.5 g NaCl for the method. Standard 1.8 mL dark glass autosampler vials were used to contain the final extracts.

Sampling

Two cities were selected in Fayoum governorate for the evaluation of four commonly used pesticides, 14 qirat in Ebshoai and 9 qirat in Sennores at May 2008. Pesticides were sprayed in two groups (Azoxystrobin &L-cyhalthrin) and (Fenhexamid & Cyproinil). The rates of spraying were 0.5cm^3 /L for azoxystrobin, fenhexamid and cyprodinil, and 1cm^3/L for l-cyhalothrin. Samples were collected from both grape and grape leaves at initial zero time, 1,4,7,10,13, 16 and 21 days beginning from 24th of May, a control sample also was taken before spraying to avoid any addition of the same pesticide residues used in application. Samples were taken carefully and representatively to all studied regions and transported into the lab. in ice box. The samples were homogenized and kept in the refrigerator at 4^{o}C for analysis.

Extraction Procedure

Quick, Easy, Cheap, Effective, Rugged and Safe method (QuEChERS) European Committee for standardization (2007) and Anastassiades *et al.* (2003) are used for the extraction and determination of the residues of the studied fungicides and insecticides .as follows: Weigh 10g (W) grapes sample in 50 ml PFTE tube, Add 10 ml acetonitrile and shake vigorously for one minute. Add Buffer-salt-mixture and shake immediately for one minute. Centrifuge the sample at 4000 rcf for 5 minutes. Transfer 4 ml of the clear solution into 50 ml round-bottomed flask and evaporate on rotary evaporator at 40^{o}C. Re-dissolve in 4 ml methanol/water (1:1) buffer solution .Inject 25µl of the sample into LC-MS/MS system.

LC-MS/MS analysis

Separation was performed on a C18 column ZORBAX Eclipse XDB-C18 4.6 x 150 mm, 5 µm particle size. The injection volume was 25 ul. A gradient elution program at 0.3 mL/min flow, in which one reservoir contained 10mM ammonium formate solution in methanol-water (1:9) and the other contained methanol was used.

The ESI source was used in the positive mode, and N_2 nebulizer, curtain, and other gas settings were optimized according to recommendations made by the manufacturer; source temperature was 400^{o}C, ion spray potential, 5500 V, decluster potential and collision energy were optimized using A Harvard Apparatus syringe pump by introducing individual pesticide solutions into the MS instrument to allow optimization of the MS/MS conditions, which are shown in Table 1. The Multiple Reaction Monitoring Mode (MRM) was used in which one MRM was used for quantification and other was used for confirmation.

Table (1) the selected multiple reaction monitoring mode used for quantification and confirmation of pesticides studied.

Pesticides	MRM1	MRM2
Azoxystrobin	404.00/372.0	404.0/148.1
L-cyhalothrin	467.22/225	467.22/141
Cyprodinil	226.0/93	226.0/169.1
Fenhexamid	302.0/97.0	302.0/288.1

RESULTS AND DISCUSSIONS

Summary of validation:

Six replicate samples for each spiking level were carried out as spiked samples for the studied pesticides at the levels of 0.01, 0.05, 0.10 and 1mg/kg. The recoveries percentages were more than 80% and the coefficient of variation CV percentages were less than 12% for six replicates for repeatability and reproducibility to each of the four studied pesticides.

Results of analysis:

Table 2 shows the concentrations of the studied pesticides in grape and grape leaves samples up to 21 days, Fig. 1a,1b,1c,1d also showed the disintegration rates of the studied pesticides and the results could be illustrated as follows:

Azoxystrubin curve in Fig. (1a) showed a regular and slow disintegration in residues beginning from 0.53 and 0.77 mg/kg in grape and grape leaf at initial time down to 0.15 and 0.45 mg/kg for grape and grape leaf respectively. Results also, illustrated that the residues in both grape and its leaf were near in concentrations and there trends of decline were linear. Azoxystrubin is a synthetic analogue of naturally occurring fungal metabolies the strobilurins and oudemansis and inhibits the mitochondrial respiration by bloking electron transfer between cytochrome b and cytochrome c at the ubiquinol oxidizing site controlling the pathogenic strains resistant of other fungicides like benzimidazoles or dicarboxamides Grasso *et al*.(2006). So, it is used as a fungicide with protectant, curative, eradicant, translaminar and systemic properties. Inhibits spore germination and mycelial growth and also, shows antisporulant activity control the germination Nita *et al.* (2007).

L-cyhalothrin is a pyrithroid pesticide, non-systemic insecticides with contact and stomach action. Fig. (1b) showed a drop of concentrations of residues in grape from 3.41 to 1.45mg/kg in the 1st day and then decline down to 0.19 mg/kg after 21 days. Similarly, residues in grape leaf dropped from 1.76 in the 1st day and continued the disintegration to 0.33 mg/kg when reached 21 days after application.

Fenhexamid residues in Fig. (1c) showed a decline rate from 2.86 at initial time to 0.46mg/kg after 21 days and declined completely of residues in grape leaf which is not detected in 21 days. Due to its novel mode of action, fenhexamid showed no cross-resistance with fungicides of other chemical groups in non-toxic to bees and other beneficial insects and thus ideally suited to fit into Integrated Pest Management programs Likas (2007).

Cyprodinil residues in Fig. (1d) showed a disintegration in residues in grape from 2.36 to 0.58mg/kg after 21 days, the concentration of grape leaf did not detected after 14 days. Cyprodinil was used as new fungicide to overcome Botryatis fruit rot caused by *Botrytis cinerea* as being as systemic product, with uptake into plants after foliar application and transport throughout the tissue in the xylem. Inhibits penetration and mycelial growth both inside and on the leaf surface Wedge *et al.* (2007).

Comparing these results of residues found with legislated Maximum Residue Limits by Codex Alemintarius Committee (CAC) and European countries in Table 3. All grape are acceptable since the MRL's of Azoxystrubin, Cprodinil and Fenhexamid established for table grape are 2, 3 and 5 mg/kg consequently, whereas in case of grape leaf for the last three fungicides were 0.05 for each of them. So, cyprodinil and fenhexamid is accepted after one week of application and azoxystrubin is not accepted according to EU MRL's which may need some washing or boiling process to lower the levels of residues. The post harvest intervals (PHI) of all the studied fungicides and insecticide were less than 21 day.

Table (2) Concentrations of azoxystrobin, l-cyhalothrin, fenhexamid and cyprodinil in mg/kg during the studied period.

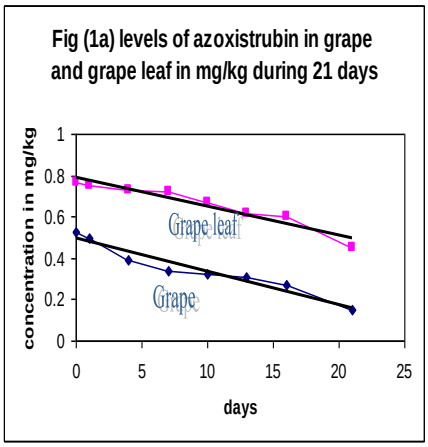
	Concentration in mg/kg of Azoxystrubin		Concentration in mg/kg of l-cyhalothrin		Concentration in mg/kg of Fenhexamid		Concentration in mg/kg of Cyprodinil	
Days	**Grape**	**Grape leaf**	**Grape**	**Grape leaf**	**Grape**	**Grape leaf**	**Grape**	**Grape leaf**
Initial	0.53	0.77	3.41	1.76	2.86	1.85	2.36	0.15
1	0.50	0.75	1.45	1.20	2.10	0.60	1.85	0.06
4	0.39	0.73	1.39	1.09	1.83	0.11	1.34	0.02
7	0.34	0.72	0.85	1.05	1.30	0.07	1.29	0.01
10	0.32	0.67	0.53	0.85	1.23	0.02	1.14	<LOQ*
13	0.31	0.62	0.48	0.75	1.13	0.01	0.94	ND**
16	0.27	0.60	0.45	0.46	1.10	<LOQ	0.83	ND
21	0.15	0.45	0.19	0.33	0.46	ND	0.58	ND

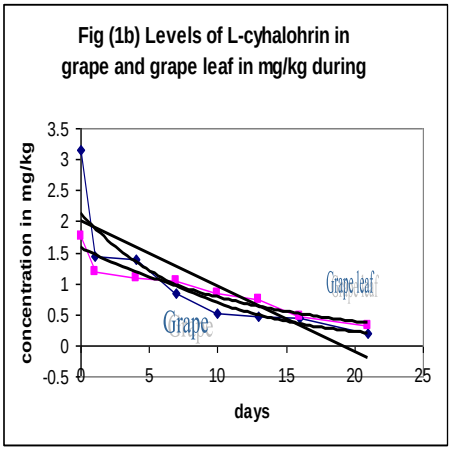
*** <LOQ is less than limit of Quantification = 0.01 mg/kg**

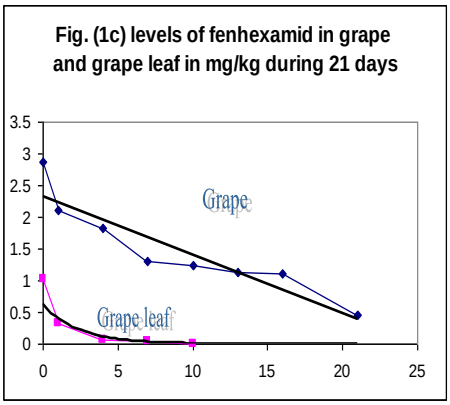
**** ND is not detected**

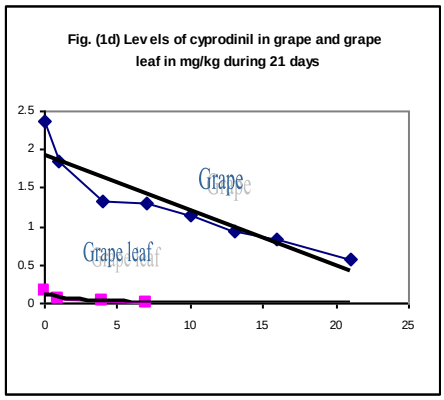
Table (3) The legislative maximum residue limits by CAC and EU countries.

Pesticide	Commodity	Maximum Residue Limits in mg/kg	
		CAC	EU
Azoxystrubin	Grape/Table	2.0	2.0
	Grape/leaf	-	0.05
Cyprodinil	Grape/table	3.0	5.0
	Grape/rasin	5.0	-
	Grape/leaf	-	0.05
Fenhexamid	Grape/table	5.0	5.0
	Grape/leaf	-	0.05









REFERENCES

Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D. and Scheneck, F. J. J.(2003).Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the determination of Pesticide Residues in Produce, J. AOAC Int.,(2003)412-431.

Ellis, M.A.;Welty, C.; Funt, R.C.; Doohan, D.; Wiiliams, R.N.and Bordelon, B. (Eds), (2004). Midwest Small Fruit Pest Management Handbook. Ohaio State University Extension, Columbus, OH pp1-234

Erincik, O.; Maden, L.V.;Ferree, D.C. and Ellis, M.A. (2001) Effect of growth stage on susceptibility of grape berry and rachis tissues to infection by *Phomopsis siticola*. Plant Dis. 85,517-520.

Grasso, V.; Palermo, S.; Sierotzki, H.; Garibaldi, A. and Gisi, U.(2007) Cytochrome b gene structure and consequences for resistance to Qo Inhibitor fungicides in plant pathogens. Pest Management Science, 62(6),pp, 465-472.

Franck, J.; Latorre.; Torres, R. and Zoffoli J. P.(2005) The effect of pre-harvest fungicide and post harvest sulfur dioxide use on post-harvest decay of table grapes caused by *Penicillium expansum*. Post-harvest Biology and Technology 37 20-30.

Likas, D.T.;Tsiropoulos,N.G. and Miliais, G.E. (2007) Rapid gas chromatographic method for the determination of famoxadone, trifloxystrubin and fenhexamid residues in tomato, grape and wine samples Journal of Chromatography A,1150 208-214

Nita, M.; Ellis M. A.; Wlison L. L. and Madden L. V. (2007) Evaluation of the curative and protectant activity of fungicides and fungicide-ajuvant mixtures on Phomopsis cane and leaf spot on grape a controlled environment study. Crop Protection 26 1377-1384.

Quick, Easy, Cheap, Effective, Rugged and Safe Method (QuEChERS) for determination of pesticide residues in foods using LC-MS-MS.(2007)

European Committee for standardization/Technical Committee 275, Standard,2007.P.EN15662.

Schilder, A.M.C.; Gillert, J.M. and Sysak, R.W. (2000) Evaluation of fungicides for control of Phomopsis cane and leaf spot of grape. 1999. FN. tests55,105

Wedge, D.E.; Smith,B.J.; Quebedeaux, J. P. and Constantin, R.J. (2007). Fungicide management strategies for control of strawberry fruit rot diseases in Louisiana and Mississippi. Crop protection 26 1449-1458.

ثبات بعض المبيدات علي ثمار وأوراق العنب

اميل يوسف سلامه

المعمل المركزي لتحليل متبقيات المبيدات العناصر الثقيله فى الأغذيه - مركز البحوث الزراعية

تـم أختيارأربعـة مركبـات كيمائيـة ثـلاث مـنهم مبيـدات فطريـة وهـم الأذوكسيسـتروبين والفينهيكساميد والسيبرودينيل ومبيد حشرى وهو السايهالوثرين لدراسة مدى ثباتهم على محصول العنب ومعرفة الفترة الآمنة التى يتم فيها جمع المحصول بعد استخدام تلك المبيدات.كما تم تطبيق هذة الدراسة فى مدينتى سنورس وابشواى بمحافظة الفيوم على مساحتين٩ و١٤ قيراط وتم تجميع عينات ممثلة بدءاً من بعد الرش مباشرة و١٦،١٣،١٠،٧،٤،١ و٢١ يوماً على التوالى من محصول العنب وأوراقه.

اظهرت الدراسة ان مركب الأذوكسيستروبين قد قلت كمية المتبقى منة من ٠,٥٣ و٠,٧٧. مج/كجم الى ٠,١٥ و٠,٤٥فى محصول العنب وأوراقة على التوالى وأيضا أنحدر كمية المتبقى من مركب السايالوثرين من ٣,٤١ الى ٠,١٩ مجم/كجم و١,٧٦ إلى ٠,٣٣مجم/كجم فى محصول العنب وأوراقة بعد ٢١ يوما من الرش وأوضحت الدراسة ايضا تناقص كمية المتبقى من مركب الفينكساميد من ٢,٨٦ الى ٠,٤٦ ولم يتواجد المبيد بعد ٢١ يوما فى الاوراق بعد ان كانت كميته بعد الرش مباشرة ١,٨٥ مجم/كجم اما مبيد السيبرودينيل تدهورت كمية المتبقى منه من ٣٦.٢ الى ٠,٥٨ مجم/كجم بعد مدة الدراسة . وإضمحلت كمية المتبقى فى الأوراق بعد ٢١ يوما وقدرت بـ ١,٨٥ بعد اليوم الأول .

وبمقارنة بيانات المتبقى المتواجد وبعد ٢١ يوما من الرش بالحدود القصوى المسموح بها عالميا سواء لجنة الكودكس أو لجنة الأتحاد الأوروبى فقد وجد أن كل هذة الكميات مسموح بها الا فقط فى أوراق العنب الخاصة بالأذوكسيستروبين التى قد تحتاج ألى بعض العمليات لأزالتها مثل الغسيل والسلق.