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RESIDUAL BEHAVIOUR OF FENARIMOL AND FLUSILAZOLE FUNGICIDES IN GRAPES

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ABSTRACT

Fenarimol and flusilazole residues on and in grape leaves were detected at all intervals, but not detectable at day 22 for fenarimol and day 29 for flusilazole on and in fruits of grape after spraying. The initial deposits of fenarimol and flusilazole on and in grape leaves (0.610 and 0.230 ppm) were higher than the initial residues on and in fruits of grape (0.050 and 0.048 ppm). The results also indicated that the dissipation of fenarimol and flusilazole on the fruits of grape (RL_{50}s: 0.6 and 0.86 days) were faster than on the grape leaves (RL_{50}s: 2.7 and 8 days).

In this experiment these pesticides had low initial residues below the Codex MRLs. Only initial residue for fenarimol on grape leaves was higher than the Codex MRL. According to the MRLs and the residual amounts of fenarimol and flusilazole in grape leaves and fruits of grape, approximate waiting time values (preharvest intervals) were 3 and 1 days and 1 and 1 days, respectively.

Data revealed that boiling process was 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, respectively.

Key word : Residual , Fenarimol , Flusilazole , Grapes

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INTRODUCTION

Grapevine *Vitis vinifera* is one of the main summer fruit crops and an important commodity in Egypt. Its leaves are also commonly used in Egypt for human consumption as stuffed grape leaves. In Egypt this crop is attacked by many fungal diseases which cause serious injury and thus the final yield is reduced.

Fenarimol [(±) -2,4'-dichloro-∝-(pyrimidin-5-yl) benzhydryl alcohol] is a systemic fungicide with protective, curative, and eradicant action and also translocated acropentally within the plant. Flusilazole [bis(4-fluorophenyl) (methyl) (1*H*-1,2,4-triazol-1-ylmethyl) silane] is a broad spectrum systemic fungicide with protective, curative action, and effective against many pathogens. According to Corda *et al.* (1993) Velsteke *et al*. (1994), Balamuralikrishnan and Jeyarajan (1998), Navarro *et al*. (2001), and indi *et al*. (2001) these pesticide leave their residues on the treated plants. It is advisable to have information on pesticide residues on and in plants under the local conditions. Such information are needed while monitoring the use of pesticides in pest control and the consumption of the economic plant product.

The objectives of the present study were to estimate the residues of fenarimol and flusilazole in grapevine plants (leaves and fruits). The safe interval between application and marketing as well as the efficiency of blanching process in reducing these pesticide residues from grape leaves were determined.

MATERIALS AND METHODS

1. Experimental and pesticide treatments

The field-work was carried out in the experimental parcels of land in a 18 year-old plantation of vineyards. The experiment plot was situated (32 vine stocks/parcel), and the treatment were carried out one time. Three experimental plots at Tahnan-Kaha-Kalubia governorate, Egypt were used. Fenarimol (Rubgan 12% EC) and flusilazole (punch 40% EC) were applied on June 28, 2003, at rates of 10 and 3 ml per 100 liter water (recommended dose), respectively, using portable motorized sprayer. One parcel was left untreated as a control check and for recovery purposes. Replicate samples, 300 g of grape leaves

and 500 g fruits of grape were taken at intervals of one hour after application (initial time), 1, 3, 5, 8, 12, 16, 22 and 29 days.

Grape leave samples were collected at intervals: initial, 1 and 3 days and were divided into two parts, the first was left unprocessed (fresh grape leaves) and the second was blanched with boiling water for three minutes to determine the effect of blanching process on removal of residues of pesticides used, then drained and left until they reached room temperature. All samples were kept in polyethylene bags in deep freezer until analysis.

2. Analytical procedures

A.1. Fenarimol pesticide:- The method of Mollhoff (1975) was adopted for extraction of fenarimol from grape leaves and fruits of grape, methanol was used instead of acetone. Fifty grams of grape leaves and 100 g of fruits were placed in the blender cup and a constant amount of methanol (2 ml/gram plant material) were added, then blended for three minutes and filtered. Extracts were shaken successively with 100, 50 and 50 ml of methylene chloride in separatory funnel after adding 40 ml of sodium chloride solution (20%); then the water phase was discarded. The combined methylene chloride phases were dried by filtration through anhydrous sodium sulphate. Then, it was evaporated just to dryness using a rotary evaporator at 40^{o}C.

A.2. Flusilazole pesticide:- The chopped 50 g of grape leaves and 100 g of fruits of grape were blended with 200 ml acetone in a blender jar for three minutes at high speed and filtered. Transferred 80 ml of the filtrate into a separatory funnel to which 100 ml petroleum ether and 100 ml methylene chloride were added and shaken for one minutes. On separation, the upper organic layer was dried by passing through anhydrous sodium sulphate. The lower aqueous layer was similarly extracted two times, each with 100 ml methylene chloride after adding 7 g NaCl and the methylene chloride phases were dried as above. The combined organic and methylene chloride phases were evaporated just to dryness using a rotary evaporator at 40^{o}C (Luke *et al*., 1975).

B. Clean-up of extracts

B.1. Fenarimol extract:- The florisil column clean up procedure of PAM (1994) was employed in clean up of fenarimol extract. A column chromatographic was prepared by adding successively, a plug of glass wool and 10 g of activated florisil (60-100 mesh). The column was pre washed with 50 ml petroleum ether (40-60^{o}C) and drained the

level of the solvent down to the top of florisil. Residue extract was transferred to the florisil column with petroleum ether, then the column was eluted with 200 ml mixture of 50% diethyl ether – 50% petroleum ether (v/v) at flow rate 5 ml/min. The eluant was evaporated just to dryness as previously described and the residues were ready for GC determination after redissolved in an appropriate volume of ethyl acetate.

B.2. Flusilazole extract:- The residue was dissolved in 5 ml methanol and cleaned up according to Johnson (1963) using coagulating solution (0.5 g ammonium chloride and 1 ml 85% orthophosphoric acid solution in 400 ml distilled water). The residue was thoroughly mixed with 10 ml of cooled freshly prepared coagulating solution and the contents were quantitatively transferred and filtered through a chromatography column of 2.5 cm diameter packed with a 5 cm layer of Hyflo-super cell. Transfer was repeated three times using 5 ml methanol and 10 ml coagulating solution each. The filtrate was then collected together in 250 ml separating funnel and extracted with 3 x 50 ml methylene chloride. The extracts were collected and evaporated just to dryness as previously described and redissolved in an appropriate volume of HPLC-grade acetonitrile. Finally, the extract was filtered through a 13 mm, 0.45 μm neylen filter into a glass stopper test tube, then the residues were ready for analysis by HPLC.

C. Chromatography determination

C.1. Gas liquid chromatography determination:- GC-systems-HP 6890 Series equipped with an electron capture detector (ECD) and workstation was used to determine fenarimol. The capillary column HP-PAS 5, 25 m x 0.32 mm, with 0.25 μm film thickness (5% phenyl) – methylpolysiloxane. Operating conditions: nitrogen carrier gas 4 ml/min and temperature degrees were 300^{o}C for injection port, 250^{o}C for column, and 320^{o}C for detector.

C.2. High pressure liquid chromatography determination:- Flusilazole was determined using an Agilent Technologies Series 1100 HPLC system equipped with workstation. UV-Photodiode array detector set at 201 nm, and the analytical column Nucleosil-C18, 5 μm (4 x 250 mm) was used. The mobile phase was acetonitrile-water (70:30) at flow rate 1 ml/min and the injection volume was 20 μl.

Results were corrected according to the rates of recovery which were determined in fortified untreated samples at levels ranged from 0.1 to 1 ppm. Following the techniques previously mentioned, the rates of recovery for fenarimol and flusilazole were 84.66%, 91.73% and 80.82%, 92.34% in grape leaves and fruits of grape, respectively.

RESULTS AND DISCUSSION

Tables (1 and 2) shows residues of fenarimol and flusilazole on and in grape leaves and fruits of grape after treatments. Fenarimol and flusilazole residues on and in grape leaves were detected at all intervals, but not detectable on and in fruits of grape at day 22 for fenarimol and day 29 for flusilazole after spraying. The initial deposits of fenarimol and flusilazole on and in grape leaves were 0.610 and 0.230 ppm, whereas they recorded the lowest of initial residue (0.050 and 0.084 ppm) on and in fruits of grape, one hour after application. Residues of fenarimol and flusilazole decreased to 0.340 and 0.210 ppm on and in grape leaves with loss 44.26% and 8.69%, and 0.010 and 0.035 ppm on and in fruits of grape with loss 80% and 58.33%, respectively within the first day after spraying. The residues of these pesticides decreased gradually until reached 0.080 and 0.022 ppm on and in grape leaves with loss 86.88% and 90.43% of the intial amounts, and 0.001 and 0.003 ppm on and in fruits of grape with loss 98% and 96.42% of the initial residues, after 16 days from treatments, respectively. Also the estimated residue half-life values (RL_{50}) of fenarimol and flusilazole were 2.7 and 8 days and 0.6 and 0.86 days on grape leaves and fruits of grape, respectively.

In this experiment these pesticides had low initial residues below the Codex MRLs, except only initial residue of fenarimol on grape leaves was higher than the Codex MRL. The maximum residue limits (MRLs) of fenarimol and flusilazole in grape were 0.3 and 0.5 ppm (Codex Alimentarius Commission, 2000). According to the MRLs, and the residual amounts of fenarimol and flusilazole in grape leaves and fruits of grape, approximate waiting time values (per harvest intervals) were 3 and 1 days, and 1 and 1 days, respectively.

It is clear from the present study that the initial deposits of fenarimol and flusilazole on grape leaves were higher than on the fruits of grape, this variation probably relates to their different surface

to weight ratios and perhaps, different surface properties. In this respect, El-Sayed *et al*. (1976) stated that the amounts of deposits depend upon the rate of application, the nature of the treated surface and the relation between the treated surface and its weight. The variation in residue levels of the pesticides between the leaves and fruit tissues following application could be attributed to original differences between the surface and biochemical constituents of leaves and fruits (Al-Samariee *et al*., 1988). Also the data indicated that the dissipation of fenarimol and flusilazole on the fruits of grape (RL_{50}s: 0.6 and 0.86 days) were faster than on the grape leaves (RL_{50}s: 2.7 and 8 days). This result could be attributed to the biological dilution for fruits of grape which was higher than that of grape leaves. Christensen (2004) reported that the decline of pesticides may be due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Plant growth, particularly for fruits is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects (Walgenbach *et al*., 1991). In addition, the rapid dissipation of originally applied pesticide is depend on a variety of environmental factors such as sunlight and temperature (Lichtenstein, 1972).

Degradation data in this study indicate that fenarimol and flusilazole were more persistent in grape leaves than fruits of grape. These results coincide those reported by many investigators. Balamuralikrishnan and Jeyarajan (1998) reported that the fenarimol residues persisted in fruits of grapevine for a maximum of 14 days after the last spray (low dosage), and the dissipation rate was higher during the summer. The fenarimol residue levels on grapevines immediately after application was 0.53 mg/kg, but fell to 0.06 mg/kg 28 days and the calculated half-life time was 7.8 days (Navarro *et al*., 2001). Indi *et al*.(2001) found that the residues of triadimefon, myclobutanil, penconazole, cyproconazole and flusilazole in the treated fruits of grape after 45 days of last spray were below detectable limits. Residues levels of flusilazole in the roots of celeriac did not exceed permitted limits, but foliage levels were higher, still being detectable at last 6 weeks after treatment (Vulsteke *et al*., 1994). The behaviour of the fenhexamid fungicide on grapes has been studied by Cabras *et al*. (2001) and reported that the residue on grapes after treatment decreased rapidly to one-third of the initial level after first

week, while it remained constant during the following two weeks. Furthermore, Corda *et al*. (1993) reported that the degradation of fenarimol appeared slower in the samples of oil from treated olives collected 0, 10 and 20 days after the last treatment. Corda *et al*. (1985) also found that fenarimol and triadimefon degraded with the established safety period on globe artichoke, while degradation of imazalil was also fairly rapid. Alary *et al*. (1993) reported that the treatment of some vegetables grown in greenhouses had residues of fenarimol < less or = > equal to the WHO accepted limits .

Data in Table (1) and (2) show that boiling of grape leaves was very effective in removal of fenarimol residues than flusilazole residues, from treated grape leaves. Fenarimol and flusilazole residues decreased from 0.610 and 0.230 ppm in the fresh leaves at the initial time to 0.210 and 0.193 ppm in the boiled leaves revealing a total removal of 65.57% and 16.09%, respectively. 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 boiled leaves, respectively.

Several investigations have been carried out to evaluate the cooking and blanching processes in removing pesticide residues from grape leaves and other plant products, Hegazy *et al*. (1999) found that boiling process was very effective in eliminating dimethoate residues, but was less effective in reducing prothiofos and diniconazole residues on and in treated grape leaves. In another study, boiling process removed about 96% of paclobutrazol residues on and in grape leaves picked one day after application (Hegazy *et al*., 1988). Also, Haggag (1994) reported that the blanching process removed about 99% of triazophos residues on moloukhia leaves. Furthermore, Holland *et al*. (1994) showed that the loss of chlorothalonil by cooking in open system by volatilization was 85-98%, whereas in a closed system the loss was reduced to only 50%. Christensen *et al*. (2003) found that the significant reduction of tolylfuanid by cooking of strawberries (91% reduction) is in line with results obtained by Rasmussen *et al*. (2004) who found a 81% reduction of tolylfluanid when cooking of apples.

Table (1): Residues of fenarimol on and in fresh and boiled grape leaves and fruits of grape.

Time after application (days)	Residues					
	Leaves		Boiled leaves		Fruits	
	ppm	% loss	ppm	% Removal	ppm	% loss
Initial*	0.610	0.00	0.210	65.57	0.050	0.00
1	0.340	44.26	0.180	47.06	0.010	80.00
3	0.280	54.09	0.120	57.14	0.009	82.00
5	0.160	73.77	–	–	0.008	84.00
8	0.140	77.05	–	–	0.005	90.00
12	0.100	83.60	–	–	0.003	94.00
16	0.080	86.88	–	–	0.001	98.00
22	0.030	95.08	–	–	ND**	–
29	0.008	98.68	–	–	ND	–
RL_{50} *** in hours	2.7				0.6	
MRL**** (ppm)	0.3				0.3	
Preharvest intervals (days)	3				1	

* One hour after application.
** Not detectable.
*** Calculated from persistence curve
**** According to Codex Alimentarius Commission (2000)

Table (2): Residues of flusilazole on and in fresh and boiled grape leaves and fruits of grape.

Time after application (days)	Residues					
	Leaves		Boiled leaves		Fruits	
	ppm	% loss	ppm	% Removal	ppm	% loss
Initial*	0.230	0.00	0.193	16.09	0.084	00.00
1	0.210	8.69	0.158	24.76	0.035	58.33
3	0.159	30.86	0.008	49.69	0.027	67.85
5	0.128	44.35	–	–	0.018	78.57
8	0.115	50.00	–	–	0.010	88.09
12	0.100	56.52	–	–	0.005	94.04
16	0.022	90.43	–	–	0.003	96.42
22	0.016	93.04	–	–	0.001	98.80
29	0.008	96.52	–	–	ND**	–
RL_{50}*** in hours	8				0.86	
MRL**** (ppm)	0.5				0.5	
Preharvest intervals (days)	1				1	

* One hour after application.
** Not detectable.
*** Calculated from persistence curve
**** According to Codex Alimentarius Commission (2000)

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الملخص العربي

سلوك متبقيات مبيدي الفيناريمول و الفلوزيلازول في نباتات العنب

شكر عبد السلام علي شكر ، اسلام نعمان نصر ، هند عبد اللاه محمود
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أجريت هذه الدراسة بهدف تتبع متبقيات المبيدين الفطريين فيناريمول وفلوزيلازول على وفي اوراق وثمار العنب . حيث قُدرت متبقيات الفيناريمول والفلوزيلازول على وفي اوراق العنب عند كل الفترات التي اخذت عندها العينات خلال التجربة. ولكن لم تكتشف أي متبقيات لمبيد الفيناريمول على ثمار العنب بعد 22 يوم من المعاملة وكذلك لم تكتشف اي متبقيات لمبيد الفلوزيلازول على ثمار العنب بعد 29 يوم من المعاملة . المتبقيات الأولية لمبيدي الفيناريمول والفلوزيلازول على وفي اوراق العنب (0.610 و 0.230 جزء في المليون) كانت أعلى من القيم المقابلة للمبيدين على وفي ثمار العنب (0.050 و 0.048 جزء في المليون) . وكذلك أظهرت النتائج أيضاً أن معدل اختفاء مبيدي الفيناريمول والفلوزيلازول من على ثمار العنب (فترة نصف العمركانت 0.6 و 0.86 يوم) كان أسرع من معدل اختفاء كلا المبيدين من على اوراق العنب (فترة نصف العمركانت 2.7 و 8 ايام).

في هذه التجربة كانت المتبقيات الأولية لهذين المبيدين اقل من الحد المسموح به المحدد من قبل لجنة دستور الغذاء والدواء (الكودكس) ماعدا المتبقيات الأولية لمبيد الفيناريمول على أوراق العنب كانت أعلى من الحد المسموح به طبقاً للكودكس . وعلى ذلك يمكن اعتبار أن فترة ماقبل الحصاد عند معاملة محصول العنب بمبيد الفيناريمول هي 3 أيام بالنسبة لاوراق العنب ويوم واحد بالنسبة لثمار العنب . وكذلك عند المعاملة بمبيد الفلوزيلازول على محصول العنب فان يوم واحد بعد المعاملة يكون كافي لاستهلاك الاوراق والثمار بأمان .

كما أوضحت الدراسة ان عملية سلق اوراق العنب في الماء المغلي لمدة 3 دقائق كانت فعالة في ازالة متبقيات مبيد الفيناريمول من اوراق العنب بنسبة أعلى منها في حالة ازالة متبقيات مبيد الفلوزيلازول من على أوراق العنب عند اجراء عملية السلق .حيث كانت نسبة الازالة لمبيدي الفيناريمول والفلوزيلازول من اوراق العنب نتيجة عملية السلق بعد يوم واحد من المعاملة هي 47.06 % و 24.76 % مع انخفاض في كمية المتبقيات من 0.340 و 0.210 جزء في المليون في اوراق العنب الغير معرضة لعملية السلق الى 0.180 و 0.158 جزء في المليون في اوراق العنب التي تعرضت لعملية السلق .