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DISSIPATION OF SWITCH AND PENCONAZOLE IN GRAPES

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ABSTRACT

The disappearance of switch (cyprodinil and fludioxonil) and penconazole, which are widely used in fresh grapes leaves and fruits to control powdery mildew, downy mildew and fruits rot, was studied. Grapes were harvested for tested fungicides analysis at 1 hour 3, 6, 10, 12, 15, 21days after treatment. The determination of residues was carried out by GC- ECD. The residue levels in leaves immediately after application were 11.98, 4.31 and 3.12 mg/kg but fell to 0.01 mg/kg 21days after application for cyprodinil, fludioxonil and penconazole, respectively. While in fruits the residues were 4.31, 2.50 and 0.62 mg/kg after 1 hour and these amounts undetected after 21days from application. The recoveries of cyprodinil, fludioxonil and penconazole were 86.60, 81.76 and 83.15 for leaves. While for fruits the percentages were 90.50, 94.30 and 91%, respectively. The limits of determination of the method were 0.01 and 0.005 mg/kg for switch and penconazole, respectively. The pesticides half- life times in leaves were 3.04, 3.16 and 2.50 days for cyprodinil, fludioxonil and penconazole, respectively But in fruits were 3.53, 1.2 and 1.03 days for cyprodinil, fludioxonil and penconazole, respectively.

Key words: grapes – residues fungicides

INTRODUCTION

A grape *Vitis vinifera* is the non-climacteric fruit, botanically a true berry, that grows on the perennial and deciduous woody vines of the genus *Vitis*. Grapes can be eaten raw or used for making jam, juice, jelly, vinegar, wine, grape seed extracts, raisins, and grape seed oil. Grapes are also used in some kinds of confectionery. In Egypt the quantity exported in the year 2006 reached to 68,296 tonnes (HRI,

2008). It is important to produce high quality table grapes by producing crop with low residue levels by applying a crop protection program that optimizes the use of plant protection products according to Integrated Crop Management (ICM)/Integrated pest Management (IPM) principles. To this end, the partners have been working together first to meet the demand for high- quality table grapes don't exceed the maximum residue levels established by Codex or European Union and second to bring down the level of residues of plant protection products in the final produce without compromising crop quality.

Fungicides prevent and cure diseases which can have severe adverse effects on crop yields and quality.

Switch components (cyprodinil and fludioxonil)

Cyprodinil 4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine

Systemic product, with uptake into plants after foliar application and transport throughout the tissue and acropetally in the xylem.

Fludioxonil4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-arbonitrile

Non-systemic fungicide with long residual activity. Uptake into the plant tissues and curative properties are generally limited. Inhibits mainly the germination of conidia and, to a lesser extent, the germ tube and mycelial growth.

Penconazole-(2,4-dichloro-β-propylphenethyl)-1H-1,2,4-triazole) formulated as topas. Penconazole is of great importance for the control of some fungal disease such as Erysiphaceae Venturai spp. And other pathogenic ascomycets, Basidiomyces and Deuteromycetes, especially on cucurbit, grapes, pomefruit, ornamentals and vegetables, (Tomlin, 1997). In Egypt, penconazole is recommended for controlling powdery mildew in grape (Anonymous, 2001).

The present investigation amide to

(1) Determine the persistence of switch components (cyprodinil and fludioxonil) and penconazole residues in leaves and fruits of grape.

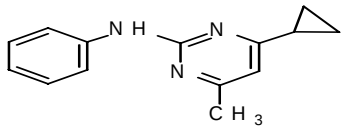
(2) Determine the pre-harvest intervals (PHI) of tested fungicide on grape.

MATERIALS AND METHODS

1. Pesticides used:

Two fungicides i.e., switch and penconazole were used in this study. Their properties, common, trade and chemical names, structure formula, formulation form and application rate are illustrated as follow:

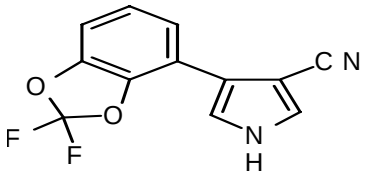
1-1-**Switch** (62.5% WG)
(a) Cyprodinil (37.5% w/w)
Structure formula:



Chemical name: 4-cyclopropyl-6-methyl-*N*phenylpyrimidin-2-amine

(b) Fludioxonil (25% w/w)

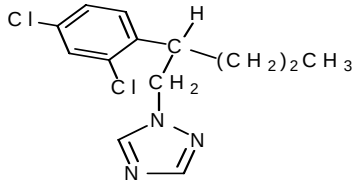
Structure formula:



Chemical name: 4-(2,2-difluoro-1,3-benzodioxol-4- yl)pyrrole-3-carbonitrile

Rate of application of switch: 50gm/100L of water

Structure formula:



1.2. Penconazole (Topas) (100% EC):
Chemicalname: 1-(2,4-dichloro-β-propylphenethyl)-1*H*-1,2,4-triazole
Rate of application: 10cm^3/100L of water

2-Pesticides application:

2-1-Field application and sampling

The field experiments were carried out in three plots at Shanessa village, Dahkahlia Governorate, Egypt. The area of each plot was 1/20 of feddan. Cyprodinil and fludioxinil (the components of switch) and penconazole were applied on 6 of August, 2008, at rate of 50gm/100L water and 10 cm^3/100 L water respectively, using a knapsack sprayer fit with a single nozzle was used. One plot was left untreated as control check and for recovery purposes.

Fresh vine leaves and grapes samples were consisted of three replicates (300 – 500 grams), were collected. A representative samples were taken one hour after application and then after 1, 3, 6, 10, 12, 15 and 21 days respectively.

The collected grape leaves were transferred to the laboratory in ice box, then homogenized and divided to three sub samples each about 50 grams then stored at -20ºC in deep freezer until residue immidatly analysis. Representative samples of grown fruits weighed 1.5 kg, which could be marketed, were randomly taken one hour after application and then after 1, 3, 6, 10, 12, 15 and 21 days respectively from the plants of each treatment. The collected grape fruits were transferred to the laboratory in ice box, removed one by one and divided into three sub -sample each about 100 grams and kept at – 20°C until pesticide residues analysis. Control samples (grape fruits and leaves) were taken from untreated plot.

3-Laboratory experiments:

After transported of leaves and grape fruits samples to the laboratory, the samples were divided into sub samples, after then, the extraction, clean up and quantitative determination were carried out as following:

3.1. Samples extraction:

The technique of extraction was mentioned by Mollhof (1975) which adapted as follow and was found to be suitable for extraction of the tested pesticide.

Fifty grams was cut into small pieces in a warning blender. Methanol was 200 ml added to sample and blended for 3min. at a high speed and filtered through dry pad cotton into a graduated cylinder. A known volume of extract was taken. For separation of water from

methanol extract, the extract was portioned successively with 100, 50 and 50ml of dichloromethane after adding 40ml of NaCl saturated solution. The combined methylene chloride was dried by filtration through a pad of cotton and anhydrous sodium sulfate, which then evaporated at 40°C.

3-2-Clean up:

The residue of cyprodinil and fludioxinil (the components of switch) and penconazole extract was dissolved in 5 ml methanol and cleaned up according to the adopted method of Johnson,(1963), using coagulating solution (0.5gm ammonium chloride and 1ml 85% orthophosphoric acid solution in 400 ml distilled water). The extract was thoroughly mixed with 10 ml of cooled freshly prepared coagulating solution and the extract was quantitatively transferred and filtered through a chromatographic columns of 2.5 cm diameter packed with a 5 cm layer of Hyflo-super cell was repeated three times using 5 ml methanol and 10 ml coagulating solution in each time. The filtrates were then collected together in 250 ml separating funnel and extracted with 3 x 50ml methylenechloride. The final extract was concentrated using rotary evaporator and dissolved in known volume of ethyl acetate for GLC analysis.

3-4- Instrumentation

Quantitative analysis of switch (cyprodinil and fludioxinil) and penconazole residues were performed using gas liquid chromatograph (GLC), HP 6890 serial equipped with electron capture detector (ECD) and capillary column HP-5 (30 m x 0.25 mm i.d x 0.25 μm film thickness). The temperatures for cyprodinil and fludioxinil were 300°C and 260°C for detector and injector, respectively. The column temperature was programmed at 220°C for 2min., and raised to 260°C at the rate of 3°C/min., and then holed for 10min. The flow rate of nitrogen carrier gas was 3.2ml/ min. The method showed linearity for all samples with a very high correlation coefficient (r = 0.999). Under the optimized GLC - ECD conditions, the retention time of cyprodinil and fludioxinil were 3.20 and 5.06 min. respectively. While for penconazole were 320°C and 300°C for detector and injector, respectively. The column temperature 230°C for 2min., and raised to 260°C at the rate of 3°C/min., and then holed for 10min. The flow rate of nitrogen carrier gas was 3ml/ min. the retention time was 3.06 min.

3-5-Recovery studies.

Known quantities of tested fungicides dissolved in acetone and added to untreated samples of different parts of grapes. Simultaneous processing frequently checked recovery of the overall method. The recovery percentages from spiked samples of grapes illustrated in table (1), for all matrices analyzed leaves and fruits. The average recovery rates of cyprodinil and fludioxinil (the components of switch) and penconazole fungicides on grape leaves were 86.60, 81.76 and 83.15 for leaves, which were 90.5, 94.30 and 91%, respectively for fruits. The results were calculated for the individual residue from the area displayed by an integrator attached to the instrument and the data were adjusted for recoveries.

Table (1): Recovery percentages of tested pesticides from leaves and fruits of grapes.

Pesticide	Recovery (%)	
	Leaves	Fruits
Cyprodinil	86.60	90.50
Fludioxinil	81.76	94.30
Penconazole	83.15	91.00

RESULTS AND DISCUSSION

Residues of cyprodinil in grape leaves and fruits after treatment (50g/100L water), at the period of one hour, 1, 3, 6, 10, 12, 15 and 21 days are depicted out in Table (2). The initial deposits of cyprodinil were 11.98 mg/kg and 4.31 mg/kg in leaves and fruits of treated grapes, respectively. The residues of cyprodinil declined to 0.01 mg/kg in leaves after 15 days of application but not detected in fruits, and it was undetectable after 21 days in leaves and fruits. The half - life values of cyprodinil were calculated to be 3.04 and 3.53 days for grape leaves and fruits, respectively. According to the maximum residue limit (MRL) value of cyprodinil on grapes (5 mg/kg) (EU, 2008), the safe harvest interval (PHI) was suggested to be 6 days for grapes. The data representing the residue levels and percent dissipation of fludioxinil on grape leaves and fruits were presented in Table (3). Such data indicate that the initial concentrations were 4.31 and 2.50 mg/kg on leaves and fruit samples, respectively, one hour after fludioxonil application. The level of fludioxonil residues on

grape leaves were 3.31, 2.27, 0.66, 0.49, 0.10 and 0.01 mg/kg after 1, 3, 6,10,12 , 15 days of application, respectively, while it was under detectable limits after 21 days. Whereas, the residue level on grape fruit samples was decreased gradually to 0.02 mg/kg after 12 days and it was not detected after 15 or 21 days of treatment.

Table (2): Cyprodinil Residue levels in grape leaves and fruits

Time after application (days)	Cyprodinil					
	Leaves			Fruits		
	Residues mg/kg	*% Loss*	*% persistence*	*Residues* mg/kg	*% loss*	*% persistence*
Initial*	11.98	0.00	100	4.31	0.00	100
1	8.43	29.63	70.37	3.75	12.99	87.01
3	6.08	49.24	50.76	2.48	42.45	57.55
6	2.34	80.47	19.53	0.66	84.68	15.32
10	1.09	90.90	9.10	0.41	90.48	9.52
12	0.06	99.50	0.50	0.01	99.76	0.24
15	0.01	99.92	0.08	ND	-	-
21	ND	-	-	ND	-	-
RL_{50} (days)	**3.04**			**3.53**		

* Samples were taken one hour after application ND : Not detected
MRL : 5 mg/kg (EU 2008)
LOD : 0.01 mg/kg
RL_{50} : Residue half-life

The half- life time values of fludioxinil were 3.16 and 1.05 days on applied leaves and fruit, respectively. The results indicate that grapes treated with fludioxonil could be consumed after 6 day of application, where the maximum residue limit (MRL) for fludioxonil in grapes is 2 mg/kg according to Codex, (1997).

The fate of new fungicides (cyprodinil, fludioxonil, pyrimethanil, and tebuconazole) from the treatment on vine to the production of wine was studied by Paolo *et al.,* (1997). They found that the fungicide residues on grapes showed different decay rates after treatment, with first-order kinetics and half-lives ranging from 8 to 57 days. The mechanism of disappearance studied with model system shows that the decrease in residues was due to codistillation

and photodegradation in fludioxonil and to evaporation and codistillation in cyprodinil Vincenzol *et al.*, (2002).

Table (3) Fludioxinil residue levels in grape leaves and fruits

Time after application (days)	Fludioxonil					
	Leaves			Fruits		
	Residues mg/kg	*% loss*	*% persistence*	*Residues* mg/kg	*% loss*	*% persistence*
Initial*	4.31	0.00	100	2.50	0.00	100
1	3.31	20.20	79.80	1.32	47.20	52.80
3	2.27	47.33	52.67	0.52	79.20	20.80
6	0.66	84.69	15.31	0.21	91.60	8.40
10	0.49	88.63	11.37	0.05	98.00	2.00
12	0.10	97.68	2.32	0.02	99.20	0.80
15	0.01	99.77	0.23	ND	-	-
21	ND	--	-	ND	-	-
RL_{50}(days)	**3.16**			**1.05**		

* Samples were taken one hour after application
ND : Not detected
MRL : 2 mg/kg (Codex 1997)
LOD : 0.01 mg/kg
RL_{50} : Residue half-life

Two fungicides (cyprodinil and fludioxonil) have recently been used in Southeast Spain to control disease in lettuce and grape. Residual values 21days after application were below the maximum residue limit (MRL = 0.05 mg/kg) established by Spanish law in the field experiment for both compounds Marin *et al*., (2003). Pesticides applied to grape vines before harvest may concentrate in the grape seed due to their high oil solubility. Residue concentrations of the fungicides such as procymidone ,iprodione , cyprodinil, fenhexamid, fludioxinil , pyrimethanil and trifloxystrobin , and the insecticides, indoxacarb and tebufenozide, were higher in grape seed oil and grape seed meal than in the fruit and the marc Gavin *et al*., (2009). Vaquero *et al*, (2008) reported that the limits of quantification were lower than maximum residue limits (MRLS) in grapes.

Grape was treated with 10 cm^3/100L water of penconazole (100% EC). The results in Table (4) show that the initial concentrations of penconazole on grape leaves and fruits were 3.12 and 0.62 mg/kg, respectively after one hour of application. The residues level was decreased to 0.01 mg/kg in leaves after 21days of treatment. Penconazole was not detected in fruit samples after 15 and 21days of application. The half -life time values of penconazole were 2.62 and 1.03 days for grape leaves and fruits, respectively. The MRL for penconazole which recommended according to Codex, (2003) on grapes is 0.20 mg/kg. Data indicate that grapes could be consumed safely after 15days from application.

Table (4) Penconazole residue levels in grape leaves and fruits.

Time after treatment (days)	Residues (mg/kg) Leaves	% Loss	% *Persistence*	Residues (mg/kg) fruits	% Loss	% *Persistence*
*Initial	3.12	0.00	100	0.62	0.00	100
1	2.21	29.16	70.84	0.32	48.38	51.62
3	1.34	57.05	42.95	0.10	83.87	16.13
6	0.87	72.11	27.89	0.07	88.70	11.30
10	0.53	83.01	16.99	0.02	96.77	3.23
15	0.12	96.15	3.85	ND	-	-
21	0.01	99.67	0.33	ND	-	-
$RL_{50\ (days)}$	2.62			1.03		

* Samples were taken one hour after application
ND: Non detected
MRL 0.20 mg/kg (Codex,2003) RL_{50}: Residue half-life

Based on the dissipation pattern of tested pesticide residues in relation to their respective prescribed maximum residue limits, PHI values are 6, 6 and 15 days suggested for grapes treated with cyprodinil and fludioxinil (the components of switch) and penconazole, respectively.

Lim *et al*., (1990) reported that the rapid disappearance of penconazole from the leaves is probably related to volatilization of penconazole. No residues of penconazole could be detected in grapes after 120 days from application. These results agreed with those obtained by Cabras and Angioni (2000). Naser *et al*., (2003) found that the fungicide incorporated into vine leaves decreased rapidly with a half –life of less than 3 days .No penconazole residues detected in

grapes picked up at marketing time after 120 days from field application.

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اختفاء مبيد السويتش و البنكونازول فى العنب

هند عبد اللاه محمود
المعمل المركزى للمبيدات- مركز البحوث الزراعية- الدقى- الجيزة

تم دراسة اختفاء مبيدى السويتش (سبيرودينيل والفلوديوكسنيل) والبنكونازول اللذين يستخدمان لمكافحة البياض الدقيقى و البياض الزغبى واعفان الثمار0حيث تم تحليل المبيدات الفطرية محل الدراسة فى كلا من أوراق وثمار العنب بعد 1 ساعة ، 3، 6، 10،12 ، 15، 21 يوم من المعاملة و كان مستوى المبيدات بعد ساعة واحدة من الرش 11.98 ، 4.31 و 3.12 جزء من المليون ولكن انخفضت الكمية الى 0.01 جزء من المليون بعد 21 يوم من المعاملة لكلا من السبيرودينيل والفلوديوكسنيل والبنكونازول بينما كانت الكميات المتبقية من الثمار 4.31 ، 2.50 و 0.62 جزء من المليون على التوالى بعد ساعة واحدة من المعاملة وهذه الكميات اختفت بعد 21 يوم من المعاملة 0كانت نسبة الاسترجاع فى الاوراق86.60 ، 81.76 و 83.12% فى كلا من السبيرودينيل والفلوديوكسنيل والبنكونازول على التوالى 0بينما كانت نسب معدل الاسترجاع فى ثمار العنب كالاتى 90.50 ، 94.30 و 91 % عى التوالى 0وكانت حدود التقدير هى0.01 و 0.005 جزء من المليون لكلا من السيويتش البنكونازول على التوالى 0كما كانت قيم فترة نصف العمر على الاوراق 3.04 ، 3.16 و2.50 يوما لكلا من السبيرودينيل والفلوديوكسنيل والبنكونازول على التوالى 0بينما قيم فترة نصف العمر على الثمار هى3.53 ، 1.20 و1.03 يوم للمبيدات المستخدمة على التوالى 0