



EFFECT OF GIBBERELIC ACID ON THE PERSISTENCE OF CERTAIN PESTICIDES ON/IN GRAPE FRUITS

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ABSTRACT

Recommended application was done by spray regime for two pesticides, namely diniconazole, fenitrothion and a plant growth regulator (gibberellic acid) and their mixtures. Their residues were determined after different successive treatments in and on grape fruits in two consecutive years. The initial deposits of the fungicide diniconazole when used alone were 0.49 and 0.50 ppm for 2006 and 2007, respectively. While the initial deposits of fenitrothion reached to 11.35 and 11.19 ppm for the same interval, respectively. Initial deposits of the plant growth regulator gibberellic acid were 30.52 and 30.42 ppm for the same period, respectively.

After mixing the tested pesticides and the plant growth regulator, the initial deposits loss reached 38.77 and 42.0 % for diniconazole, 43.08 and 48.16 % for fenitrothion and 6.88 and 7.00 % for gibberellic acid for the same seasons, respectively. A significant degradation was recorded with the mixture of the two tested pesticides and plant growth regulator compared with that occurred when pesticide was used alone at the two studied seasons.

INTRODUCTION

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production, they contain large amounts of phytochemicals which offer health benefits (Mandal *et al* 2010). Grape

crop is mainly subjected to infestation with mealy bugs, powdery mildew and berry rot. The insecticide fenitrothion (Sumithion) is recommended for controlling mealy bugs and thrips, while the triazole fungicide diniconazole (Sumi-eight) is recommended for controlling powdery mildew and berry rot according to pest control program, (Ministry of Agriculture and Land Reclamation, Egypt, 2001). Four applications of gibberellic acid (GA3) are currently used to increase the berry size of Thompson Seedless grapes. GA3 significantly improved berry size and cell elongation, it could cause corkiness of stems and delayed ripening, (Singh *et al* 1978 and Mervet *et al* 2001).

The wide use of pesticides in agriculture and their background levels in the environment could be a source of many biochemical and physiological disturbances in plants, animals and human (Mahmoud 2004). Plant growth regulators as gibberellic acid (GA3) are important in regulating the plant growth in which the formation of bunch of flower, berry set, berry enlargement, extension of bunch of grapes, dilution of berry in a bunch of grapes, preventing berry cracking, killing pollens and causing grape varieties having seeds becoming seedless, and has no harmful effect on human health. However, the application time, dose, the age, the growth period, etc. of the plant that the application is done on, are important (Korkutal *et al* 2008).

The present study aimed to investigate the persistence of diniconazole, fenitrothion, gibberellic acid and their mixtures in and on grape fruits and the preharvest interval (PHI) for each of the tested pesticides alone and in mixtures.

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MATERIALS AND METHODS

This work was carried out during 2006 and 2007 seasons on twenty years old vigorous fruitful Thompson seedless grapevines grown in private vineyard at Kalubia governorate. A triazole systemic fungicide namely diniconazole in the form of sumieight 5% EC, fenitrothion, non systemic insecticide in the form of sumithion 50% EC and a plant growth regulator as gibberellic acid in form of Berlex 10% tablet were applied according to the following mode of application.

1) Experimental Design

The cultivated areas with grapevines were divided into 5 plots, each plot contained 10 vines. The following treatments were carried out as follows:

Treatment (1): Ten vines were left without treatment as a control and for recovery test.

Treatment (2): Ten vines were treated with diniconazole at 35 ml/100 L water.

Treatment (3): Ten vines were treated with fenitrothion at 150 ml /100 L water.

Treatment (4): Ten vines were treated with gibberellic acid at 10-20-30-40 ppm, respectively as shown in Table (1).

Treatment (5): Ten vines were treated with mixtures of diniconazole, fenitrothion and gibberellic acid at the above mentioned rates.

Spray regime was done as follows: (1) three treatments for each pesticide alone, (2) Four treatments for the plant growth regulator, (3) Only one treatment for the mixture of each pesticide with the plant growth regulator, (4) Two treatments for the mixture of the two pesticides and the plant growth regulator. The initial sample was taken after the fourth spray for each pesticide alone, the plant growth regulator and the mixture of the two pesticides with the plant growth regulator (Table 1).

2) Sampling

Representative samples from grapes were taken at random after one hour, 1, 2, 5, 7, 11, 14, 19 and 23 days from treatments, for pesticides and plant growth regulator residue analysis. Sub-sampling was done, where three samples of 50 g of treated grapes were taken. Sub samples were kept in clean poly-ethylene bags and stored at -20°C in a deep freezer until the time of residue analysis.

3) Extraction and Clean up

Methanol was adopted for the extraction of diniconazole in a technique mentioned by Mallhof (1975) instead of acetone, and cleaned up using the coagulating solution used by Johnson (1963). Residues of fenitrothion were analyzed according to the technique developed by M.W.H.C.A., Netherlands (1988). The extracts of fenitrothion were cleaned up by Malhat (2006) method. The extraction technique described by Gordon and Pankratz (1968) was followed for the extraction of gibberellic acid.

4) Residues Determination

4.1. Diniconazole

Hewlett Packard 6890 series gas chromatography system equipped with electron capture detector (ECD) was used for the determination of diniconazole residues at the following operating condition using DB-17 Column (15 m×0.32 mm× 0.52 µm film thickness). The operating temperatures were 220, 320 and 320°C for the column, injector and detector, respectively, using N₂ gas as carrier gas with a flow rate of 4 ml/ min.

4.2. Fenitrothion

Quantitative analysis of fenitrothion residues were performed by a Hewlett Packard 6890 series gas chromatography system equipped with Flame photometric detector (FPD), operated in the phosphorus mode (525 nm filter under the following operation conditions: The used column was: Capillary column was P.As-1701(14%cyanoprophyl phenyl methyl polysiloxane) (30m×0.32 mm× 0.25 µm film thickness). The operating temperatures were 230, 240 and 250°C for the column, injector and detector, respectively, using N₂ gas as carrier gas with a flow rate of 4 ml /min. The hydrogen and air flow were 75 and 100ml/min

4.3. Gibberellic acid

Determination of gibberellic acid residues was done by HPLC Agilent 1100 equipped with diode array detector under the following operation conditions: Column: Zorbax SBC18 (30m×0.32 mm× 0.25 µm film thickness). The Mobile phase was: methanol/acetonitrile/water. (60/35/5.V/V/V) with a flow rate of 0.8 ml/min.

Table 1. Purpose of treatment, time of application and plant stages

Purpose of treatment	Time of application	Treatments					
		D	F	G	D+G	F+G	D+F+G
Elongation	10-13 cm ³ of cluster length	—	150 cm ³ /100 L water	10 ppm	—	✓	—
First Thinning	10% flowering	35 cm ³ /100 L water	—	10-20 ppm	✓	—	—
Second Thinning	70% flowering	35 cm ³ /100 L water	150 cm ³ /100 L water	20-30 ppm	—	—	✓
Sizing	10-12 mm of berry size	35 cm ³ /100 L water	150 cm ³ /100 L water	40 ppm	—	—	✓

G-gibberellic acid

F-fenitrothion

D-diniconazole

✓- treated with mixtures of diniconazole, fenitrothion and gibberellic acid at the recommended rates

5) Recovery Studies

Untreated samples of grape were spiked with known amounts of diniconazole, fenitrothion and gibberellic acid prior to extraction and clean up for recovery determination. These samples were passed through the entire process of extraction, then clean up and analysis as previously described. By following such techniques the rates of recovery for diniconazole, fenitrothion and gibberellic acid residues from spiked plant samples treated with 0.1, 0.5 and 1 ppm were determined. The obtained results were corrected according to the recovery rate. Data in Table (2) proved the satisfactory rate of recovery, reaching 92.08, 101.29 and 91.79 in average for diniconazole, fenitrothion and gibberellic acid, respectively.

Table 2. Recovery percent of tested agrochemicals from spiked plant samples

Fortified limit (ppm)	% Average of recovery		
	Diniconazole	Fenitrothion	Gibberellic acid
0.1	90.97	99.88	89.32
0.5	91.23	100	92.75
1.0	94.06	104	93.32
Average	92.08	101.29	91.79

RESULTS AND DISCUSSION

1) Persistence of diniconazole and its mixtures on & in grape fruits

The dissipation of any compound depends on various factors, including plant species, chemical formulation, climatic conditions, physical phenomenon mainly volatilization, application method and chemical conditions in which sunlight play prominent role Mandal *et al* (2010).

Results in Table (3 and 4) indicated that the three pesticides when used alone showed higher residue levels than those recorded with their mixtures at most intervals during the two seasons. The initial deposits found after one hour from the fourth spray at 2006 were 0.49 ppm (diniconazole alone) and 0.30 ppm for its mixture with the two other pesticides with percent of loss difference 38.77. On the other hand, the initial deposits at 2007 were 0.50 ppm for diniconazole alone and 0.29 ppm for its mixture, with loss difference of 42.0 % at the same interval.

The estimated half-life ($t_{1/2}$) values for diniconazole alone were 22.49 hrs and 25.32 hrs for its mixture on & in grape fruits at 2006, which reached 23.03 and 24.78 hrs, respectively at 2007.

According to Codex Alimentarius Commission (2010), the maximum residue limits for diniconazole on/in grape fruits was 0.05 ppm.

The corresponding recommended pre-harvest intervals (PHI) were 7.0 and 5.0 days in the two seasons after application of diniconazole alone (D) and of its mixture (M-D), respectively. These results are in agreement with those of **Hegazy et al (1999)** who studied the residues of diniconazole on/in grape leaves and found that the safety period that should be waited before marketing grape leaves is at least three weeks to reach its MRL level (0.2 ppm).

2) Persistence of fenitrothion and its mixtures on/in grape fruits

Data presented in **Tables (3 and 4)** indicated that fenitrothion when used alone showed higher persistence compared to its mixtures at all intervals during the two studied seasons. The initial deposits one hour after the fourth spray in 2006 were 11.35 ppm for fenitrothion alone and 6.46 ppm for its mixture, showing a loss of 43.08 %. On the other hand, the initial deposits in 2007 reached 11.19 ppm (fenitrothion) and 5.80 ppm (its mixtures) with a loss of 48.16 %.

The $t_{1/2}$'s for fenitrothion alone were 28.23 hrs and 20.15 hrs for its mixture on/in grape fruits in 2006, compared with 26.72 and 19.21 hrs in 2007, respectively.

The level of pesticides residue is affected by many factors, i.e. applied rate, meteorological factors, biological aspects in addition to the nature and properties of the plant surface. The obtained results are in coincide with those reported by **Hegazy et al (1988-a)** and **Hegazy et al (1997a & b)**.

According to **Codex Alimentarius Commission (2010)**, the MRL's for fenitrothion on/in grape fruits was (0.1 ppm). The obtained pre-harvest intervals (PHI's) were 9.0 and 7.0 days at the two seasons after application for fenitrothion alone (F) and its mixture of fenitrothion (M-F), respectively. The rapid disappearance of fenitrothion may be due to its high vapor pressure (18 mPa at 20 °C). Similar findings were recorded by **Hegazy et al (1997-b)** and **Hegazy et al (1999)**.

Generally, it was found that organophosphorus pesticides were degraded within short periods in plants and other environmental constituents (**Al-Samariee et al 1988**). The safe period for harvesting the organophosphorus insecticides treated vegetables ranged between 1 and 12 days post-treatment, depending on the chemistry of tested pesticide and kind of crop **El-Sayed et al (1977)**.

3) Persistence of gibberellic acid (GA3) and its mixtures on/in grape fruits

The results given in **Tables (3 and 4)** indicated that gibberellic acid alone showed more persistence than its mixtures within all intervals during the two seasons.

The estimated half-life ($t_{1/2}$) values for gibberellic acid alone was 17.15 hrs compared to 13.69 hrs for its mixture on/in grape fruits at 2006, which reached 17.11 and 13.68, respectively at 2007.

Such data revealed that GA3 showed the greatest and fast degradation compared with the other two pesticides. According to (**M.H.W.S.) Netherlands (2006)**, the maximum residue limits for GA3 on/in grape fruits was 5.0 ppm. The obtained (PHI's) were 4.0 and 2.0 days at the two seasons after application of GA3 alone (G) and its mixture (M-G), respectively.

In this regard, **Hegazy et al (1988-b)** reported that paclobutrazol in grape leaves showed fast degradation during the first day after application, followed by slow decomposition through the experimental period until disappearance on/in grape leaves. This short persistence in/on grape fruits could be attributed to a variety of environmental factors such as sunlight and temperature **Lichtenstein (1972)**.

Besides, plant growth is also responsible to certain extent for decreasing the pesticide residue concentrations due to growth dilution effect (**Walgenbach et al 1991**). However, the degradation rates gradually decreased through the elongation of post-treatment interval.

The above results are in agreement with those obtained by **Abdel-Hamid (2008)** as they studied some environmental factors on the decomposition of diniconazole, fenitrothion and gibberellic acid after different intervals from exposure. The half life value in case of single pesticide revealed less value than that in case of mixing with the other compounds which emphasizes rapid decomposition rate of the tested compounds when mixed together.

From **Tables (3) and (4)**, we found that the initial deposit of the tested pesticides alone was much higher than that of the pesticides mixtures, which may be attributed to the physical properties of the pesticides. **Abdel-Hamid (2008)** found that fenitrothion, diniconazole and gibberellic acid had lower pH, conductivity, salinity, surface tension and viscosity with respect to the same pesticide in the mixture. She proved a strong relationship between pesticide residues and physical properties of spray solution.

Table 3. Residues of Diniconazole, Fenitrothion, Gibberellic acid (GA3) and their mixtures after application on/in grape fruits during season 2006

Intervals (days)	D		M-D			F		M-F			G		M-G		
	ppm	% loss	ppm	% loss	% Differences*	ppm	% loss	ppm	% loss	% Differences*	ppm	% loss	ppm	% loss	% Differences*
Initial	0.49	0.00	0.30	0.00	38.77	11.35	0.00	6.46	0.00	43.08	30.52	0.00	28.42	0.00	6.88
1	0.23	53.06	0.16	46.66	30.43	6.85	36.64	2.25	65.17	67.15	10.30	66.25	7.35	74.14	28.64
2	0.10	79.59	0.09	70.00	10.00	2.60	77.09	0.49	92.41	81.15	5.69	81.35	3.70	86.98	34.97
5	0.07	85.71	0.05	83.33	28.57	0.91	91.98	0.14	97.83	84.61	1.82	94.03	0.00	100.00	-
7	0.04	91.84	0.03	90.00	25.00	0.14	98.77	0.09	98.60	35.71	0.00	100.00	0.00	100.00	-
11	0.03	93.88	0.01	96.67	66.67	0.04	99.64	0.01	99.84	75.00	0.00	100.00	0.00	100.00	-
14	0.00	100.00	0.00	100.00	-	0.00	100.00	0.00	100.00	-	0.00	100.00	0.00	100.00	-
t _{1/2} (hours)	22.49		25.32			28.23		20.15			17.15		13.69		
MRL (ppm)			0.05					0.1					5		
PHI (days)	7		5			9		7			4		2		

D: Diniconazole alone.

M-D: Diniconazole mixed with fenitrothion and gibberellic acid.

F: Fenitrothion alone.

M-F: Fenitrothion mixed with diniconazole and gibberellic acid.

G: Gibberellic acid alone.

M-G: Gibberellic acid mixed with diniconazole and fenitrothion.

% Differences*: between the residues of pesticide alone and its mixture

Table 4. Residues of Diniconazole, Fenitrothion, Gibberellic acid (GA3) and their mixtures after application on/in grape fruits during season 2007

Intervals (days)	D		M-D			F		M-F			G		M-G		
	ppm	% loss	ppm	% loss	% Differences*	ppm	% loss	ppm	% loss	% Differences*	ppm	% loss	ppm	% loss	% Differences*
Initial	0.50	0.00	0.29	0.00	42.00	11.19	0.00	5.80	0.00	48.16	30.42	0.00	28.29	0.00	7.00
1	0.24	52.00	0.10	68.90	58.33	6.26	44.05	1.59	72.58	7.46	10.15	66.63	7.24	74.40	28.66
2	0.12	79.00	0.07	75.80	41.66	2.40	78.55	0.45	92.24	81.25	5.60	81.59	3.56	87.41	36.42
5	0.08	84.00	0.05	82.75	37.50	0.83	92.58	0.10	98.27	87.95	1.76	94.21	0.00	100.00	--
7	0.05	90.00	0.02	93.10	60.00	0.15	98.65	0.08	98.62	46.66	0.00	100.00	0.00	100.00	--
11	0.02	96.00	0.008	97.24	60.00	0.04	99.64	0.01	99.82	75.00	0.00	100.00	0.00	100.00	--
14	0.00	100.00	0.00	100.00	--	0.00	100.00	0.00	100.00	--	0.00	100.00	0.00	100.00	--
t _{1/2} (hours)	23.03		24.78			26.72		19.21			17.11		13.68		
MRL (ppm)	0.05					0.1					5				
PHI (days)	7	5				9	7				4	2			

D: Diniconazole alone.

M-D: Diniconazole mixed with fenitrothion and gibberellic acid.

F: Fenitrothion alone.

M-F: Fenitrothion mixed with diniconazole and gibberellic acid.

G: Gibberellic acid alone.

M-G: Gibberellic acid mixed with diniconazole and fenitrothion.

% Differences*: between the residues of pesticide alone and its mixture

Tawfik and El-Sisi (1987) and El-Sisi *et al* (1995) reported that decreasing the pH values and increasing the viscosity of the insecticide spray solution caused increase in the initial deposits and decrease in the degradation rate.

Consequently, the mixture of GA3 and the two studied pesticides could be recommended to decrease the initial deposits of GA3 and two the pesticides in the mixture as well as their residues on/in grape fruits.

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تأثير حامض الجبريلليك على ثبات بعض المبيدات فى وعلى ثمار العنب

[٢٨]

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الموجز

بعد ان تم الخلط المبيدات المختبرة ومنظم النمو، وجد أن معدل الخفض للراسب الابتدائى وصل الى ٣٨,٧٧ و ٤٢,٠٠ % لمبيد الداينيكونازول عنه بعد الخلط مع المبيدات الأخرى على التوالي لعامي ٢٠٠٦ و ٢٠٠٧. ووجد ان معدل الخفض كان ٤٣,٠٨ و ٤٨,١٦ % فى نفس الفترة لمبيد الفينثروثيون ، على التوالي. اما فى حالة منظم النمو حامض الجبريلليك فكانت ٦,٨٨ و ٧,٨٨ % لنفس الفترة. وجد ان خلط المبيدات المختبرة يؤدي الى تدهور المبيدات عن استخدامها منفردة خلال الموسمين المتعاقبين.

تم تطبيق بعض المبيدات الموصى بها بنظام الرش العادى بالرشاشة الظهرية على نباتات العنب. تم اختيار المبيد القطرى الداينيكونازول والمبيد الحشرى الفينثروثيون ومنظم نمو النبات البيريلكس (حمض الجبريلليك (GA3)). اظهرت النتائج المتحصل عليها ان الراسب الابتدائى وصل الى ٠,٤٩ و ٠,٥٠ جزء فى المليون لمبيد الداينيكونازول منفردا فى ٢٠٠٦ و ٢٠٠٧، على التوالي. بينما كان الراسب الابتدائى لمبيد الفينثروثيون ١١,٣٥ و ١١,١٩ جزء فى المليون فى نفس الفترة على التوالي. بلغ الراسب الابتدائى لمنظم نمو النبات ٣٠,٥٢ و ٣٠,٤٢ جزء فى المليون لحمض الجبريليك لنفس الفترة.