

RESIDUAL BEHAVIOUR OF CERTAIN PESTICIDES ON AND IN GRAPE LEAVES

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Abstract

The persistence of the pesticides, prothiofos (Tokuthion 50% EC), dimethoate (perfekthion 40% EC) and diniconazole (sumi-eight 5% EC), on and in grape leaves was studied. Grape leaves are commonly used in Egypt in human consumption. Grape vines, var. King Robby, were sprayed with the recommended rate of each pesticide on June 10, 1996. The estimated half-life values (RL50) of the applied pesticides were 17, 23, 27 hours for prothiofos, dimethoate and diniconazole, respectively.

Data revealed that dimethoate was the most degradable pesticide compared to the other two pesticides. Its residues on and in grape leaves decreased to a level below its Codex MRL ten days after spraying with total loss of 98.6%. Prothiofos was the most persistent pesticide in this study. Its residues level on and in grape leaves was significantly high (26.67 ppm) ten days after application and should not be used or marketed for human consumption. Although diniconazole showed low levels of residues on and in grape leaves, ten days was not enough to reduce its residues in the leaves to its MRL level. The pre-harvest interval (PHI) should not be less than three weeks.

The results also indicated that boiling process was very effective in eliminating dimethoate residues on and in treated grape leaves. The reduction of its residues due to this process was about 98%. The boiled grape leaves can be marketed one day after dimethoate treatment. On the contrary, boiling of treated grape leaves was less effective in reducing prothiofos or diniconazole residues. The reduction in their residues after this process were 35% for prothiofos and 30-50% for diniconazole.

INTRODUCTION

Grape (*Vitis vinifera*) is one of the major summer fruit crops in Egypt. Its leaves are also commonly used in human food as stuffed grape leaves by filling them with rice and ground beef. This fruit crop is mainly subjected to infestation with mealy bugs and powdery mildew. The insecticides prothiofos (Tokuthion) and formthion (Anthio) are recommended for controlling mealy bugs, while the fungicide diniconazole (sumieight) is recommended for controlling powdery mildew. Since for-

mothion is rapidly hydrolyzed in the presence of water at pH 3-9 (23°C) forming dimethoate in the spraying solution (Anonymous, 1994), the later was used in this study instead of formothion.

The present study was carried out to evaluate the residual behaviour of prothiofos, dimethoate and diniconazole on and in grape leaves under the Egyptian field conditions to determine the pre-harvest intervals (PHI), the safety periods that must be waited after application of each pesticide before picking and marketing treated grape leaves which have been exposed to great amounts of the spray solutions and may be picked for human consumption few days after pesticide applications. The effect of boiling the treated grape leaves on the removal of residues of the applied pesticides was also evaluated.

MATERIALS AND METHODS

Pesticides Used

1. Prothiofos: O-2,4-dichloro O-ethyl-S-propyl phosphorodithioate, O.P. insecticide known commercially as Tokuthion. The formulation Tokuthion 50% EC, produced by Bayer AG, Germany, was used at the rate of 150 ml/100 litres of water (i.e. 75 g ai/100 litres of water).

2. Dimethoate: O,O-dimethyl-S-methylcarbamoyl (methyl) phosphorodithioate, O.P. insecticide with contact and systemic action against a broad spectrum of insects, known commercially as Perfekthion, Roger and Roxion. The formulation Perfekthion 40% EC produced by BASF, Germany, was used at the rate of 75 ml/100 litres of water (i.e. 30 g ai/100 lit. water).

3. Diniconazole : (E)-(RS)-1-(2,4-dichlorophenyl)-4,4- dimethyl-2- (1H-1,2,4-triazol-1-yl) pent-1-en-3-ol, a triazole systemic fungicide protective against a broad spectrum of fungal diseases on cereals, peanuts, fruits and vegetables. Known commercially as Sumi-eight and Spotless. The formulation Sumi-eight 5% EC produced by Sumitomo Chemical, Japan, was used at the rate of 35 ml/100 litres of water (i.e. 1.75 g ai/100 lit. water).

Field experiment and sampling : The field work was carried out in the Experimental Farm of the Horticulture Research Institute at Giza. Grape seedlings, variety King Robby were planted in 1991. The tested pesticides were applied one time each at the recommended rates mentioned before for each pesticide using a Knapsack

sprayer on June 10, 1996. Three plots of four vines were sprayed for each pesticide and three plots were left unsprayed to serve as control.

Representative grape leaf samples were picked for each pesticide treatment an hour after spraying (zero time or initial) and at intervals of 1, 3, 6 and 10 days after application. Three replicates of leaf samples were collected for each treatment including control. Fresh samples were put in clean bags and taken to the Central Agricultural Pesticide Laboratory for residues analysis. Each sample of grape leaves of the initial and 24 hrs after spraying were divided into two portions. One portion was analyzed as fresh leaves and the other portion was boiled in water for three minutes before analysis to determine the effect of boiling on removal of pesticide residues. Samples of the following intervals were analyzed as fresh leaves. All fresh and boiled leaves samples were kept in the freezer at -20°C until analysis.

Pesticide residues analysis techniques

Extraction

Prothiofos, dimethoate and diniconazole residues were extracted from grape leaves using the method of Mollhoff (1975). The extraction was adapted by using methanol instead of acetone. Fifty grams of representative grape leaves were cut into small pieces and blended with 100 ml methanol for 3 minutes in a waring blender. The extract was filtered through a bed of washed cotton into a 250 ml graduated cylinder. One hundred milliliters of the filtrate was transferred into 250 ml separatory funnel and 40 ml of 20% sodium chloride solution was added. The mixture was extracted with 50 ml chloroform. Extraction was repeated three more times using 30 ml portion chloroform each. Extracts of chloroform were collected together in a 250 ml round bottom flask and evaporated under vacuum on a rotary evaporator at 35°C to dryness. Residues were redissolved in 5 ml methanol.

Clean up

Extracts of prothiofos were cleaned up through activated florisil using the elution solvent system of methylene chloride, n-hexane and acetonitrile at the ratio of 50: 48.5 : 1.5, respectively.

Extracts of dimethoate residues were cleaned up according Junson (1963) using coagulating solution (0.5 g ammonium chloride and 1 ml 85% phosphoric acid solution in 40 ml distilled water). The residues, dissolved in 5 ml methanol, were

thoroughly mixed with 10 ml of fresh prepared coagulating solution and the content was quantitatively transferred and filtered through a chromatography column 2.5 cm diameter packed with a 2.5 cm layer of Hyflo-Super cel and repeated twice using 5 ml methanol and 10 ml of coagulating solution each. The filtrate was then collected and transferred into a 250 ml separatory funnel and extracted with 30 ml chloroform. Extraction was repeated with 3 x 20 ml chloroform. Extracts of chloroform were collected together in a 250 ml round bottom flask and concentrated under vacuum on a rotary evaporator to about 2 ml and transferred into a glass stoppered test tube. The evaporation was completed to dryness and the residues were then redissolved in the proper volume of ethyl acetate for quantitative determination.

Diniconazole extracts were cleaned up using ammonium chloride coagulating solution according to the adapted method of Blinn and Bayd (1964). The residues were dissolved in 5 ml methanol and thoroughly mixed with 10 ml of coagulating solution prepared as mentioned above. The content was cooled in an ice bath for few minutes and then filtered through a 5 mm layer of Hyflo-Super cel on Whatman No.1 filter paper in a 3 cm diameter Buchner funnel using a vacuum pump. The content was washed three times with 5 ml methanol followed by 10 ml coagulating solution each. The aqueous filtrate was then extracted with 25 ml chloroform. The chloroform layer was passed through absorbent cotton and anhydrous sodium sulfate to a 250 ml round bottom flask. Extraction was repeated 3 x 25 ml chloroform. Extracts of chloroform were collected and evaporated under vacuum on a rotary evaporator to dryness. The residues were redissolved in a known volume of ethyl acetate for chromatography determination.

GC Analysis

Residues of prothiofos and dimethoate were detected and determined using a Pye Unicam 4500 gas chromatograph equipped with flame photometric detector (FPD) at the phosphorus mode and a 1.5 m x 4 mm i.d. Pyrex glass column packed with 4% SE-30 + 6% OV-210 on gas chromosorb Q 80-100 mesh. The established operating conditions of the GC were as follow:

Oven (column) temperature: 240°C.

Injection port temperature : 243°C.

Detector temperature: 245°C.

Carrier gas (N₂), hydrogen and air flow rates were 30 ml/min. For all.

Retention times were 6.70 and 2.49 min. for prothiofos and dimethoate, re-

spectively. The minimum detection limits were 0.002 ppm for prothiofos and 0.001 ppm for dimethoate. The average recovery percentages using this procedure were 96% for prothiofos and 93% for dimethoate.

Residues of diniconazole were determined using a Hewlett Packhard GC 8953 equipped with electron capture detector (ECD) and an HP 608 capillary column (30 m length x 0.53 mm I.D., and film thickness 0.5 μm). The established operating conditions for the GC were as follows:

Inlet temperature: 280°C, Column temperature: 260°C, Detector temperature: 330°C and N_2 Flow rate : 2.4 ml/min.

RESULTS AND DISCUSSION

A. Persistence of the applied pesticides on and in grape leaves

Table 1 shows residues of prothiofos, dimethoate diniconazole on and in grape leaves at different intervals after pesticide treatments. Prothiofos showed the highest levels of residues on and in grape leaves at all intervals, whereas diniconazole recorded the lowest of residue deposits. Dimethoate, on the other hand, showed intermediate residue levels. The initial deposits of prothiofos residues (an hour after spray) on and in the leaves was 210.75 ppm compared with 136.98 and 4.26 ppm for dimethoate and diniconazole at the same interval, respectively. The extreme amounts of the pesticide residues as well as the great variation between their deposits could be attributed to the large surface areas of the exposed grape leaves in proportion to their weight for the amounts of the detected residues, and to the differences in their applied recommended rates. The concentrations of spraying solutions were 750,300 and 17.5 ppm (as active ingredients) for prothiofos, dimethoate and diniconazole, respectively.

Regarding residues deterioration through the intervals after application, dimethoate was the most degradable pesticide compared to the other two pesticides. Its initial deposits was 136.98 ppm which decreased to reach 1.88 ppm revealing a total loss of 98.6% of the initial amount ten days after treatment. This residue level was lower than the Codex MRL of dimethoate on vegetables (2.0 ppm), which means that grape leaves treated with dimethoate at the mentioned rate can be safely used in human consumption.

Prothiofos was the most persistent pesticide in this study. Its residues on and

Table 1. Residues of the pesticides Prothiofos, Dimethoate and Diniconazole on and in fresh and boiled grape leaves at different intervals after pesticide applications.

Intervals after application	Prothiofos				Dimethoate				Diniconazole			
	Fresh leaves Residues (ppm)	Loss (%)	Boiled leaves* Residues (ppm)	Loss (%)	Fresh leaves Residues (ppm)	Loss (%)	Boiled leaves* Residues (ppm)	Loss (%)	Fresh leaves Residues (ppm)	Loss (%)	Boiled leaves* Residues (ppm)	Loss (%)
Initial (1 hour)	210.75	00	135.02	35.9	136.98	00	3.54	97.4	4.26	00	2.98	30.0
24 Hours	62.77	70.2	41.14	34.5	64.82	52.6	1.23	98.1	2.30	45.5	1.15	50.0
3 Days	41.39	80.4			28.53	79.2			1.34	68.5		
6 Days	31.56	85.6			8.28	93.9			0.76	82.2		
10 Days	26.67	87.3			1.88	98.6			0.41	90.6		
The estimated half life value												
Codex MRL'S								23 hours				27 hours
Concentration of spray solution (a.i)								2.0 ppm (on vegetables)				0.2 ppm on grapes (France)
								300 ppm				17.5 ppm

* Leaves were put in boiling water for 3 minutes before residue analysis.

in grape leaves decreased from 210.75 ppm at the zero time to reach 26.67 ppm ten days after application revealing total loss of 87.3% of the initial deposits. Although the Codex MRL of prothiofos is not established yet, its detected residue level on grape leaves seems to be extremely high and the treated leaves at this interval should not be marketed for human consumption.

In spite of the low levels of diniconazole residues on and in grape leaves at the different intervals including the initial deposits (4.26 ppm), ten days was not enough as a safety period to reduce its residues in the treated grape leaves to its MRL level (0.2 ppm) or below (The residues were 0.42 ppm at ten-day intervals). The safety period that should be waited before marketing grape leaves previously treated with diniconazole should be at least three weeks to ensure that the leaves contain residue levels below its MRL.

The estimated half-life values (RL50) of the applied pesticides were 17, 23 and 27 hours for prothiofos, dimethoate and diniconazole, respectively. This short persistence in grape leaves could be due 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 next intervals.

In this regard, Abdel Rahman (1996) sprayed some organophosphorus insecticides on lettuce, and his results indicated that prothiofos was the most persistent insecticide in comparison with the other applied O.P. compounds. In another residue study of paclobutrazol in grape leaves, Hegazy et al., (1988) reported that the compound showed fast degradation during the first day after application followed by slow decomposition through the experimental period until disappearance on and in grape leaves.

B. Effect of boiling process on reducing pesticide residues from treated leaves

The reduction of pesticide residues on and in treated grape leaves subjected to boiling water was demonstrated in Table 1. The data show that boiling of grape leaves was very effective in removal of dimethoate residues from treated grape leaves. Its residues decreased from 136.98 ppm in the fresh leaves at the initial to 3.54 ppm in the boiled leaves revealing a total loss of 97.4%. The reduction of dimethoate residues in the leaves due to boiling process was 98.1% for the leaves picked

one day after spraying. The residues decreased from 64.82 ppm in the fresh leaves to 1.23 ppm in the boiled leaves which was below its MRL (2.0 ppm). So, the PHI for the boiled leaves, previously treated with dimethoate its only one day.

On the contrary, boiling treated grape leaves was less effective in reducing prothiofos and diniconazole residues. The reduction percentages of their residues after boiling process were about 35% for prothiofos and between 30-50% for diniconazole revealing that prothiofos and diniconazole are more thermal persistent than dimethoate.

In this respect, Haggag (1994) reported that the blanching process removed about 99% of triazophos residues on moloukhia leaves. Also, boiling process removed about 96% of paclobutrazol residues on and in grape leaves picked one day after application and the PHI was one day for washed and boiled leaves, while it reached 30 days for unwashed fresh leaves (Hegazy et al., 1988).

REFERENCES

- 1 . Abdel Rahman, A.A.M. 1996. Determination of organophosphorus insecticide residues in some vegetables and detection of drainage water pollution with insecticides. M.Sc Thesis, Fac. of Agric., Cairo Univ., Giza, Egypt.
- 2 . Anonymous. 1994. The pesticide Manual, 10th Edition, British Crop Protection Council and The Royal Society of Chemistry, C. Tomlin, ed., Crop Protection Publications, The Bath Press, Bath, UK.
- 3 . Blinn, R.C. and J.E. Boyd. 1964. Colourimetric determination of residues of the dithiolane insecticide in cottonseed and cotton foliage. *J. Assoc. O. Agric. Chem.*, 47:1106-11.
- 4 . Haggag, M.N. 1994. Studies on contamination in some vegetables. M.Sc. Thesis, Fac. of Agric. Zagazig Univ., Egypt.
- 5 . Hegazy, M.E.A., M.M. Abu-Zahw, Salwa M.A. Doghuem and M.M. Diab. 1988. Residues of growth regulator cultar in fruits and leaves of vine crop. *Annals of Agric., Moshtohor, Zagazig Univ., Egypt.*
- 6 . Junson, D.P. 1963. Determination of seven insecticide residues in fruit and vegetables. *J. Assoc. O. Agric. Chem.*, 46:234-37.
- 7 . Lichtenstein, E.P. 1972. Environmental factors affecting fate of pesticides. *Nat. Acad. Sci., Nat. Res. Council Report, USA.*
- 8 . Mollhoff, E. 1975. Method for gas chromatographic determination of residues of Tokuthion and its oxon in plants and soil samples. *PflanzenschutzNachrichten Bayer*, 28:382-87.
- 9 . Walgenbach, J.F., R.B. Leidy and T.J. Sheets. 1991. Persistence of insecticides on tomato foliage and implications for control of tomato fruitworm. *J.Econ. Entomol.*, 84: 978-86.

سلوك متبقيات بعض مبيدات الآفات فى وعلى أوراق العنب

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أجريت دراسة لتتبع متبقيات المبيدات الحشريين بروثيوفوس (توكثيون) و دايميثويت (برفكثيون) والمبيد الفطرى دينيكوتازول (سومى - أيت) على وفى أوراق العنب (صنف كنج روى) بعد معاملة الشجيرات بالمعدلات الموصى بها لهذه المبيدات بتركيزات ٧٥ ، ٢٠ ، ١٠، ٧٥ ، ١٠ جرام مادة فعالة / لتر ماء على الترتيب ولمرة واحدة فى ١٠ يونيو ١٩٩٦. كانت فترات نصف العمر لمتبقيات هذه المبيدات على وفى أوراق العنب هى ٢٧، ٢٢، ١٧ ساعة للبروثيوفوس والدايميثويت والدينيكوتازول على الترتيب.

كما أوضحت الدراسة أن مبيد الـدايميثويت كان أكثر المبيدات الثلاثة تحلما، وقد انخفضت متبقياته على وفى أوراق العنب الى مستوى أقل من الحدود القصوى المسموحة لمتبقياته على الخضر وذلك بعد عشر أيام من المعاملة ، حيث وصلت نسبة الفقد فى المتبقيات الى ٩٨,٦% من الكمية المقدرة بعد الرش مباشرة. أما مبيد البروثيوفوس فكان أكثر المبيدات الثلاثة ثباتا واستمرت مستويات متبقياته فى أوراق العنب عالية بدرجة واضحة حتى بعد عشرة أيام من المعاملة (٢٦,٦٧ جزء فى المليون). وبالرغم من أن مبيد الدينيكوتازول أظهر مستويات متبقيات منخفضة نسبيا إلا أنه بعد فترة عشرة أيام من الرش لم تكن كافية لخفض متبقياته على وفى أوراق العنب إلى مستوى الحدود القصوى المسموحة من متبقيات هذا المبيد ويجب ألا تقل فترة الانتظار عن ثلاثة أسابيع بعد المعاملة.

وكذلك أوضحت الدراسة أن عملية سلق أوراق العنب فى الماء المغلى لمدة ثلاث دقائق كانت فعالة جدا فى إزالة متبقيات مبيد الـدايميثويت من أوراق العنب بنسبة خفض بلغت ٩٨% وكانت فترة ما قبل الحصاد هى يوم واحد فقط لأوراق العنب التى تعرضت لعملية السلق، بينما لم يكن لعملية السلق تأثير فعال فى إزالة متبقيات مبيد البروثيوفوس والدينيكوتازول حيث بلغت نسبة الخفض فى متبقياتها فى أوراق العنب المسلوق ٣٥% للبروثيوفوس و ٣٠ - ٥٠% للدينيكوتازول.