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Quantitative Determination of Chlorpyrifos and Penconazole Residues in Grapes Using Gas Chromatography/Mass Spectrometry

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Abstract: Samples of grape leaves and berries (CV: Zeini) were taken from grape-vine yards treated with chlorpyrifos (Dursban®) and penconazole (Ofir®) in order to determine their residues in these organs. The effect of time after spraying and number of sprays on the residues of both pesticides was studied. Gas chromatorgraphy /mass spectrometry (GC/MS) was used to determine the residue levels. Results obtained have indicated the presence of both pesticides in the tested leaf samples, but chlorpyrifos residues were detected in larger quantities than penconazole residues. Also, larger quantities of both pesticides were detected in the samples of berry cortex than in the samples of berry flesh. However, the residues of both pesticides in washing water of treated berries were lower than that in the cortex or in the flesh. This may indicate the systemic action of pesticides especially penconazole, since washing the treated berries with water two weeks after application of the last spray did not remove the pesticides from berries. Overall results have indicated that the determined quantities of both pesticides residues especially in the cortical tissues of treated berries following the sixth application of both pesticides were higher than the quantities reported by other authors in grape berries, but they were generally less than the maximum residue limits (MRLS) defined by the residue legislations in other countries.

Key words: Ofir®, Dursban®,penconazole,chlorpyrifos,grape, residues, gas chromatography, mass spectrometry

INTRODUCTION

Grape production is considered the second fruit crop in Palestine following olive production since it covers an area of about 90,000 dunums and extends over four agriculturally important districts: Hebron, Bethlehem, Ramalla and Jericho[1]. Many insect pests and diseases are yearly observed attacking grapevine yards during the growing season of which grape berry moth (*Endopzie viteana*) and grape powdery mildew (*Uncinulla necator*) are the most serious under local conditions. Large quantities of pesticides are yearly applied for control of these insect, pests and diseases. For example, application of chlorpyrifos (Dursban®) against *Endopzie Viteana* and penconazole (Ofir®) against *Uncinulla necator*[2,3]. Due to the intensive application of these two pesticides (at least six applications per growing season), a considerable quantities of their residues are usually expected to be present, especially in harvested grape berries.

To the best of our knowledge, there were no attempts to quantify the residues of these pesticides in grape-vine yards or in grape berries in Palestine, but many attempts to quantify them were reported in other countries. Navarro *et al.*,[4], for example, determined chlorpyrifos and penconazole residues in grape berries harvested at 2 h and 1, 3, 7, 14 and 28 days after treatment. The residues levels immediately after application were 6.91 and 0.14 mg kg^{-1} for chlorpyrifos and penconazole, respectively, but these levels decreased to 0.14 and 0.03 mg kg^{-1} after 28 days of their application, respectively. Also, Mustafa *et al.*,[12]; Song *et al.*,[5], Liapis *et al.*,[1]; Correia *et al.*, [7] and Fernandez *et al.*,[8] developed certain analytical methods such as GLC, SPME-GC-MS, etc. for determination of residues of ten pesticides including Penconazole and Chlorpyrifos in grapes, peaches and cucumbers. Zambonin *et al.*,[9] also developed an effective analytical method for the determination of Triazole fungicide residues (e.g. penconazole) in strawberry and wine samples. Therefore, the first objective of the present research was to determine the residual quantities of chlorpyrifos and penconazole in samples of grape leaves and berries from garpe-vine yards in Palestine. The second objective was to compare the determined residues of both pesticides with the maximum residue limits defined by other countries according to the national and international legislations in order to evaluate the spraying programs of grape-vines in Palestine.

MATERIALS AND METHODS

Chemicals and materials used: Chlorpyrifos, penconazole

and methamidophos analytical standards were purchased from Riedel-de Haes. Methamidophos was used as an internal standard. Ethyl acetate and anhydrous sodium sulphate were of analytical grade. Stock standard solutions of chlorpyrifos and penconazole (50 mg L^{-1} each) and methamidophos internal standard solution (50 mg L^{-1}) were prepared in ethyl acetate.

Field equipment: Sprayer with plastic drum (20 L capacity), in addition to protective clothes were used during the spraying.

Gas chromatography/ mass spectrometry (GC/ MS): the GC/ MS with selected ion monitoring (QP5000, SHIMADZU Corporation. Japan) was used. It was supported with auto injector (AOC-17), Class 5000 software and capillary column DB-SMS (5%- phenyl) Methylopolysiloxane 0.25 μm film thickness, with 30 meters length and 0.25 mm I.D. (Available from J and W SCIENTIFIC).

Chromatographic analysis was performed as described by Oliva *et al.* (1999) where injector was set up at 250°C, GC/MS interface at 280°C, helium carrier gas at a flow rate of 6.2 mL^{-1} min at 25°C. The sample (5 μL) was injected in the splitless injection mode. The oven temperature was programmed as follows: 90°C for 1 min, raised to 210°C (30°C^{-1} min), then to 240°C (10°C/min) and then to 280°C (5°C^{-1} min) and held for 7 min.

Field experiments

Grape-vine orchards used: Orchards of grape-vine located at Bit Eiba village near Nablus city (Palestine) was used in this study. These orchards have moderate climate during grape-vine growing season (average seasonal temperature was 26°C and average relative humidity was 55%). There was good water–holding capacity for the soil in which the grapevines are grown.

Sampling procedure: Grape-vines (CV: Zeini) that were characterized by juicy, medium-sized berries suitable for consumption and processing were used in the present work. Protective sprays with chlorpyrifos and penconazole were applied to protect the vines from powdery mildew infection and grape fruit moth attack during growing the season. Each grapevine in the orchard was treated every 2 weeks with penconazole pesticide (50 mg L^{-1} of spray solution) starting at the beginning of growing season (unfolding of the leaves from their buds).

During the early fruit ripening, Penconazole was then mixed with chlorpyrifos (50 mg L^{-1} penconazole and 0.96 mg L^{-1} chlorpyrifos) then sprayed once every 2 weeks. Fruit and leaf samples were picked up at four intervals: 14 days after the fifth spray, 1, 9, 14 days after the sixth spray. The samples were kept in the refrigerator at 2-4°C in order to be analyzed for the residues of both pesticides by gas chromatography /mass spectrometer. In addition, samples from fruits were washed with tap water and the washing water were stored in the refrigerator at 2-4°C for being analyzed for the residues. Each sample was replicated three times and then used for calculation of the mean value of pesticide residue level.

Extraction Procedure

Extraction of chlorpyrifos and penconazole from leaves, flesh and cortex of grape berries: Fifty-gram samples of leaves or fruits were blended for 10 min with 50 g of anhydrous sodium sulphate and 100 mL ethyl acetate. The solution was filtered through Buchner funnel and then was evaporated to dryness using water bath (70°C). The dry residues was dissolved in 2 mL of ethyl acetate (containing 1 mg L^{-1} of methamidophos as internal standard) and transferred into a 2 mL vial kept at -30°C until being injected into the GC/MS.

Extraction of chlorpyrifos and penconazole from washing water solution of treated berries: About 500 g of berries were soaked in about 200 mL of water for 10 min. Washing water solution was transferred into a 500 mL separatory funnel and 100 mL of ethyl acetate were added. The two liquids were shaked for 2 min. The organic layer was separated from the mixture and about 2 g of anhydrous sodium sulphate were added to the organic layer, then shaked for about 2 min in order to remove any traces of water that may be present in the organic layer. This solution was evaporated to dryness using water bath (70°C). The dry residues were dissolved in 2 mL of ethyl acetate (containing 1 mg mL^{-1} of methamidophos as internal standard) and then transferred into a 2 mL vial kept at -30°C until injected into the GC/MS.

Gas chromatographic/mass spectrometric analysis: The concentrates containing penconazole and chlorpyrifos were analyzed using gas chromato graphy/mass spectrometry with selected ion monitoring mode. The obtained results were compared with the results obtained for standards of penconazole and chlorpyrifos analyzed under the same conditions.

RESULTS

Retention time of penconazole and chlorpyrifos: The identification of chlorpyrifos and penconazole was

realized by the retention times obtained when standard solutions of concentrations between 0.2 and 5 mg L^{-1} were injected into the gas chromatograph. The Selected ion-monitoring mode (SIM) was used in the present work. Scan mass range was 40-300; SIM: penconazole m/z: 115, 137, 159, 172, 186, 201, 213, 248, chlorpyrifos m/z: 97, 125, 169, 197, 208, 258, 286, 314 and methamidophos internal standard m/z: 63, 78, 94, 108, 126, 141. In Table 1, absolute and relative retention times to methamidophos used as internal standard are presented.

Typical GC/MS chromatogram of a mixture of standards a containing chlorpyrifos and penconazole (2 mg L^{-1} each) and methamidophos (2 mg L^{-1}) as internal standard is presented in Fig. 1.

Typical mass spectra of chlorpyrifos, penconazole and methamidophos analyzed following the procedure recommended by Oliva *et al.*[10] are presented in Fig. 2 a,b and c, respectively.

Effect of number of sprays on penconazole residues in grape: The effect of number of sprays using 50 mg kg^{-1} penconazole solution in each spray application on the residues of this fungicide in grape leaves, in flesh and cortex of the fruit have been studied. The obtained results (Table 1) indicate that penconazole residues in the three parts of the grapevine after 14 days of spraying increased by increasing the number of sprays ($0.27x10^{-3}$ to $0.44x10^{-3}$ mg kg^{-1} in flesh part, $2.46x10^{-3}$ to $12.04x\ 0^{-3}$ mg kg^{-1} in fruit cortex and $2.74x10^{-3}$ to $12.04x10^{-3}$ mg kg^{-1} in leaves).

Effect of time after spraying on the penconazole residues in grape: The obtained results (Table 2) on the effect of time after spraying with penconazole solution on the residues of this fungicide indicated that the residues in different parts of the grape-vine decreased by the increase in the time after spraying ($19.09X10^{-3}$ mg kg^{-1} after 1 day to $12.04x0^{-3}$ mg kg^{-1} after 9 days and to $9.93x10^{-3}$ mg kg^{-1} after 14 days of the sixth spray on leaves). This is an indication that the penconazole residues decrease by increasing time after spraying. This decline 14 days after spraying on leaves was calculated to be 48%, while it was 93% in the cortex and in the flesh of berries.

Table 1: Retention times (n=3), absolute and relative to the internal standard (methamidophos)

Pesticide	t_R absolute (min)	t_R relative (min)	RSD (%)
Methamidophos	4.268	1.000	0.68
Penconazole	8.976	2.103	093.00
Chlorpyrifos	9.709	2.275	0.52

Table 2: Penconazole residues (mg kg^{-1}) in grape leaves and fruit after 6 applications of the fungicide spray on grapevine yards grown in Beit-Eba, Nablus during the 2002/2003 growing season (average seasonal temperature 26°C and relative humidity 55%)

Sample No.	Application No.	Sample Type	Time in days after spraying	Penconazole residues $X10^{-3}$ (mg kg^{-1})*
6	5th	Flesh Part	14	0.27
7	5th	Cortex	14	2.46
8	5th	Leaves	14	2.74
1	6th	Leaves	1	19.09
3	6th	Flesh part	1	6.29
4	6th	Cortex	1	176.63
10	6th	Cortex	9	25.70
11	6th	Flesh part	9	3.60
16	6th	Leaves	9	12.04
12	6th	Leaves	14	9.93
13	6th	Flesh part	14	0.44
14	6th	Cortex	14	12.04

*Average of three measurements

Table 3: Chlorpyrifos residues (mg kg^{-1}) in grape leaves and fruit after 6 applications of the insecticide spray on grapevine yards grown in Beit-Eba, Nablus during the 2002/2003 growing season (average seasonal temperature 26°C and relative humidity 55%

Sample No.	Application No.	Sample Type	Time in days after spraying	Chlorpyrifos residues $X10^{-3}$ (mg kg^{-1})*
6	5th	Flesh Part	14	0.39
7	5th	Cortex	14	53.27
8	5th	Leaves	14	15.01
1	6th	Leaves	1	106.47
3	6th	Flesh part	1	7.14
4	6th	Cortex	1	196.39
10	6th	Cortex	9	175.21
11	6th	Flesh part	9	4.11
16	6th	Leaves	9	64.03
12	6th	Leaves	14	51.56
13	6th	Flesh part	14	1.19
14	6th	Cortex	14	157.03

* Average of three measurements

Table 4: Determination of penconazole and chlorpyrifos residues in washing water solution of treated berries

Sample No.	Application No.	Time in days after spraying	Penconazole residues $X10^{-3}$ (mg kg^{-1})*	Chlorpyrifos residues $X10^{-3}$(mgkg^{-1})*
5	5th	14	0.06	0.08
2	6th	1	3.11	11.11
9	6th	9	0.08	0.33
15	6th	14	0.04	0.05

* Average of three measurements

Effect of number of sprays on chlorpyrifos residues in grape: The effect of number of sprays using 0.96 mg kg^{-1} chlorpyrifos solution on the residues of this insecticide in leaves, flesh and cortex of the fruit have been studied. The obtained results (Table 3) indicate that chlorpyrifos residues in the leaves and in flesh and cortex of fruit after 14 days of spraying also increase by the increase in the number of sprays ($0.39x10^{-3}$ to $1.19x10^{-3}$ mg kg^{-1} in flesh part, $53.27x10^{-3}$ to $157.03x10^{-3}$ mg kg^{-1} in the fruit cortex, $15.01x10^{-3}$ to $51.56x10^{-3}$ mg kg^{-1} in leaves.

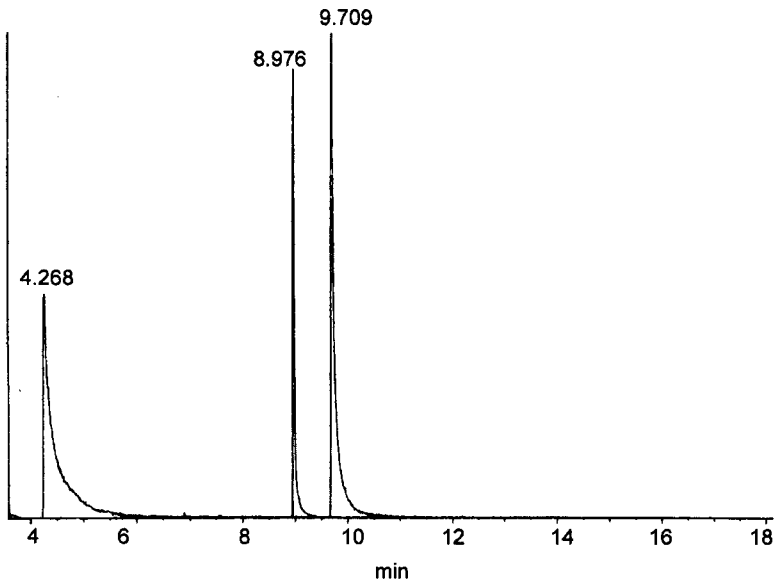

Fig. 1: Typical GC/MS chromatogram of a mixture of standards containing chlorpyrifos (9.709 min), penconazole (8.976 min) and methamidophos (4.268 min) as internal standard (2 mg L^{-1} each)

Effect of time after spraying on the chlorpyrifos residues in grape: The results obtained on the effect of time after spraying with chlorpyrifos solution on the residues of this insecticide (Table 3) indicate that the residues in different parts of the grape-vine decreased by the increase in the time after spraying ($106.47x10^{-3}$ mg kg^{-1} after 1 day to $64.03x10^{-3}$ mg kg^{-1} after 9 days and to $51.56x10^{-3}$ mg kg^{-1} after 14 days of the sixth spray on leaves). This is an evidence that the chlorpyrifos residues decrease by increasing time after spraying. This decline 14 days after spraying on leaves was calculated to be 52%, while it was 83 and 20% in cortex and flesh of the fruit, respectively.

Determination of penconazole and chlorpyrifos residues removed by washing water solution of treated berries: The residues of these pesticides in washing solution that resulted from washing sprayed grape berries with water have been studied. The obtained results (Table 4) indicate that the concentration of penconazole and chlorpyrifos removed by washing water solution did not increase by the increase in the number of sprays (almost same residual quantities 14 days after the fifth and sixth sprays). On the other hand, the concentrations of both pesticides decreased by increasing the time after spraying (from 1 to 14 days after applying the sixth spray). This clearly indicates that the penconazole and chlorpyrifos pesticides could be absorbed by the fruit while only small quantities of both pesticides can be washed by water.

DISCUSSION

It is well known that systemic pesticides including penconazole penetrate the surface of treated tissues (including the top waxy or waxy-like layers) and then move to the inside. This could explain the presence of high levels of these pesticides in the grape leaves and in fruit cortex. The obtained results are in good agreement with those reported by Navarro *et al.*,[4] on the determination of chlorpyrifos and penconazole in cortical tissues of grape berries since they reported that chlorpyrifos and penconazole residues were 0.14 mg kg^{-1} and 0.03 mg kg^{-1}, respectively after 28 days of the treatment compared to our results on the residues of both pesticides in the fruit cortex ($157.03x10^{-3}$ mg kg^{-1} for chlorpyrifos and $12.04x10^{-3}$ mg kg^{-1} for penconazole). Comparison between the concentrations of chlorpyrifos and penconazole in different parts of the grape (leaves, fruit flesh and fruit cortex) showed higher residues of chlorpyrifos in the three parts of grape organs. This is an evidence that chlorpyrifos molecule is more persistent than penconazole molecule, at least, in grape berries. These results are in good agreement with the results obtained by Garcia *et al.*,[4] who reported that chlorpyrifos was highly detected (84.9%) in the grape samples tested 10 days after maceration of treated grape berries.

The concentration of chlorpyrifos and penconazole in the washing water solution of treated berries decreased

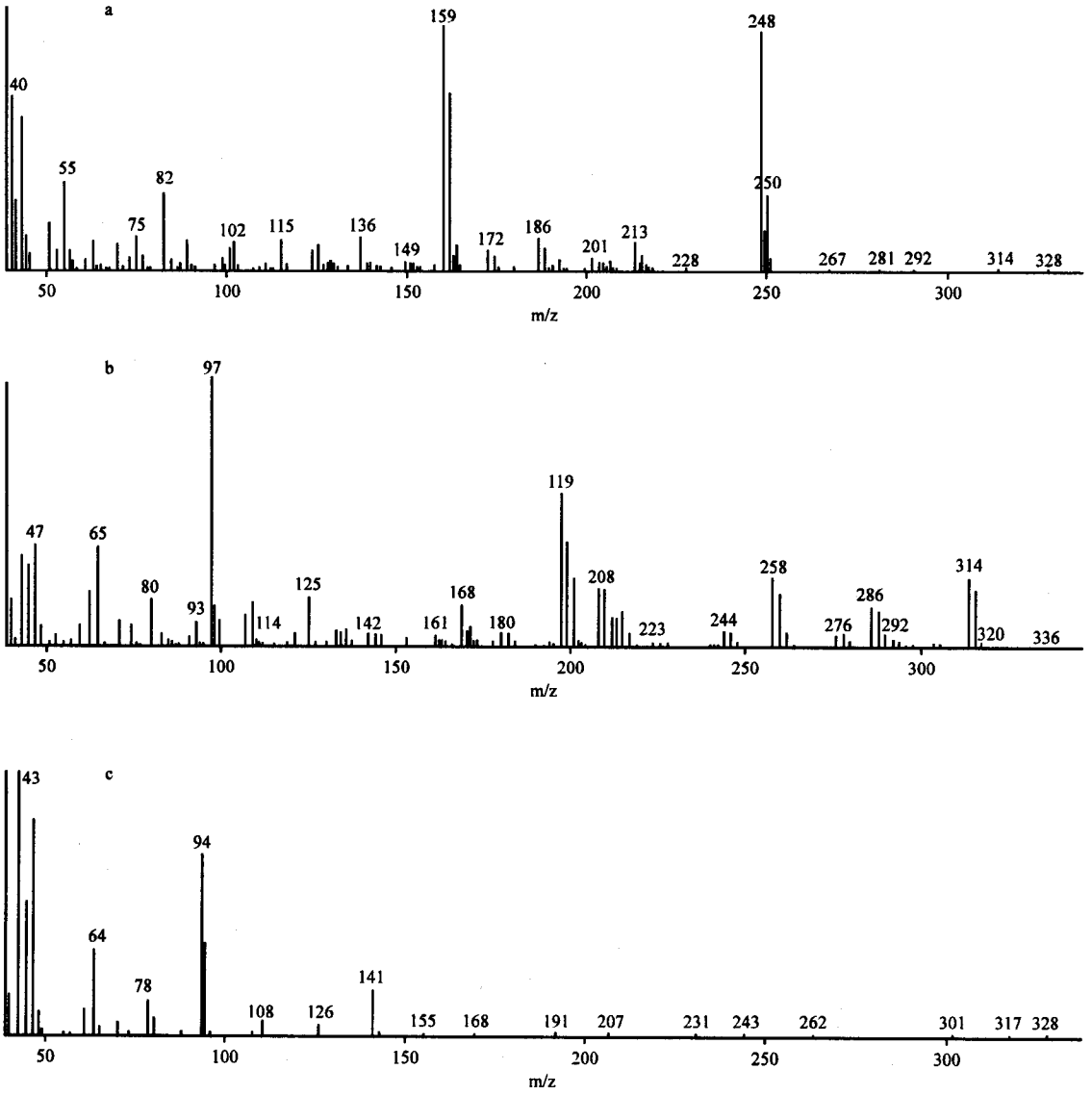

Fig. 2: Typical mass spectra of standard: (a) penconazole, (b) chlorpyrifos, (c) methamidophos

with increasing the time after the spray application. It is very important therefore to emphasis on washing the grape berries before serving when berries are recently treated with both pesticides and on the safety time recommended by health organizations for both pesticides before consuming the treated berries.

Our results showed high residues of both pesticides in the cortex of the grape fruit due to absorption of these pesticides. In order to decrease the risk of taking high concentration of both pesticides during eating fresh berries or after processing of grape fruit, it is important to respect the time needed for degradation of these pesticides due to the action of internal metabolic processes depending on the time passed after spraying. The levels of chlorpyrifos and penconazole residues that were obtained in this study in grape leaves and fruit (cortex and flesh) are almost in all cases, lower than Maximum Residues Limits (MRLs) established by different legislations in other countries (e.g. in Spanish and EU legislation, MRLs is 0.5 mg kg^{-1} for chlorpyrifos and 0.2 mg kg^{-1} for penconazole in grapes [10]. Although the residue levels found in this study are close to those found in other studies, usage of pesticides in our grape-vine yards should not be increased. Using other non- pesticide control measures against grape

powdery mildew and berry moth could therefore compensate this increase. Integrated pest management (IPM) including rational application of the pesticides might be the proper solution to the problem of these insects and diseases.

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