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Faculty of Graduate Studies

**Detection of Chlorpyrifos and Penconazole Residues in
Grape Leaves and Fruit by Gas Chromatography/ Mass
Spectrometry**

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Dedication

**To my beloved parents, brothers and sisters
With love and respect**

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List of Contents

Title	Page No.
Dedication	III
Acknowledgments	IV
List of Contents	V
List of Tables	VII
List of Figures	VIII
List of Abbreviations	IX
Abstract	X
Chapter I Introduction	1
1. Background	2
1.1. Status of grapevine production in Palestine	3
1.2. Varieties of cultivated grapes	3
1.3. Pests and diseases attack grapevines	4
1.3.1. Pests	4
1.3.1.1. Annual weeds	4
1.3.1.2. Insects	4
1.3.1.2.1. Phyloxera	4
1.3.1.2.2. Grape thrips	4
1.3.1.2.3. Grape fruit moth	4
1.3.2. Diseases	5
1.3.2.1. Powdery Mildew	5
1.4. Status of pesticides in Palestine	7
1.5. Pesticides used in this study	9
1.5.1. Penconazole (Ofir®)	9
1.5.2. Chlorpyrifos (Dursban®)	10
1.5.3. Quantitative determination of Chlorpyrifos and Penconazole	13
1.5.4. Objective of the research	17
Chapter II Materials and Methods	18
2.1. Pesticide standard solution used in the study	19
2.1.1. Chlorpyrifos standard solution	19
2.1.2. Penconazole standard solution	19
2.2. Equipment	19
2.2.1. Field equipment	19
2.2.2. Laboratory equipment	20
2.3. Field Experiment	20
2.3.1. Grapevine orchard used	20
2.3.2. Sampling procedure	20
2.4. Extraction Procedure	21
2.4.1. Extraction of Chlorpyrifos and Penconazole from Leaves Flesh and Cortex of grape berries	21
2.4.2. Extraction of Chlorpyrifos and Penconazole from washing water of treated berries	22

2.4.3. Gas chromatographic/ mass spectrometry analysis	22
Chapter III Results	23
3.1. Retention time of Penconazole and Chlorpyrifos	24
3.2. Effect of number of sprays on Penconazole residues in grape	30
3.3. Effect of time after spraying on Penconazole residues in grape	32
3.4. Effect of number of sprays on Chlorpyrifos residues in grape	34
3.5. Effect of time after spraying on Chlorpyrifos residues in grape	36
3.6. Determination of Penconazole and Chlorpyrifos in residues washing water solution	38
Chapter IV Discussion and Conclusion	40
References	43
Arabic abstract	٤

List of Tables

	Table	Page No.
Table 1.1	Area (in dunums) cultivated with grapes in Palestine	3
Table 3.1	Penconazole (Ofir®) residues (in ppm) in grape leaves and fruit after 6 applications of the fungicide spray on grapevine grown in Beit- Eba, Nablus during he growing season (average seasonal temperature of 26° C and relative humidity 55 %)	30
Table 3.2	Chlorpyrifos (Dursban®) residues (in ppm) in grape leaves and Fruit after 6 application of the insecticide spray on grapevine grown in Beit- Eba, Nablus during the growing season (average seasonal temperature 26°C and relative humidity 55 %)	34
Table 3.3	Determination of penconazole and chlorpyrifos residues in washing water solution of treated berries	38

List of Figures

	Figure	Page No.
Figure 1.1	Grape leaf infected with powdery mildew	6
Figure 1.2	Grape fruit infected with powdery mildew	6
Figure 1.3	Disease cycle of grape powdery mildew	7
Figure 3.1	GC/MS spectrum of 100 ppm of Chlorpyrifos standard solution	25
Figure 3.2	GC/MS spectrum of 100 ppm of Penconazole standard solution	26
Figure 3.3a	Typical GC/MS spectrum of grape cortex sample analyzed by using the recommended Procedures	27
Figure 3.3b	Typical GC/MS spectrum of grape leaves sample analyzed by using the recommended Procedures	28
Figure 3.3c	Typical GC/MS spectrum of grape flesh sample analyzed by using the recommended Procedures	29
Figure 3.4	Effect of number of sprays on Penconazole (Ofir®) residues (in ppm) in grape leaves and fruit using 50 mg /L Penconazole residues solution	31
Figure 3.5	Effect of time (in days) after the 6 th spray application on Penconazole residues in grape leaves and fruit (concentration:50 mg /L Penconazole solution)	33
Figure 3.6	Effect of number of sprays on Chlorpyrifos (Dursban®) residues (in ppm) in grape leaves and fruit (concentration: 0.96 mg / L Chlorpyrifos solution)	35
Figure 3.7	Effect of time (in days) after the 6 th spray application on Chlorpyrifos (Dursban®) residues (in ppm) in grape leaves and fruit (concentration: 0.96 mg / LChlorpyrifos solution)	37
Figure 3.8	Determination of penconazole (Ofir®) and Chlorpyrifos (Dursban®) residues) in washing water solution of grape berries	39

List of Abbreviations

GC / ECD: Gas Chromatography / Electron Capture Detector

GC / MS: Gas Chromatography /Mass Gas spectrometry

GLC: Gas Liquid Chromatograph

IPM: Integrated Pest Management

MRLs: Maximum Residues Limits

NPD: Nitrogen Phosphorus Detector

RSD: Residues.

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Abstract

An orchard of grapevine was used to determine the residues of chlorpyrifos (Dursban®) and penconazole (Ofir ®) pesticides during the growing season 2003. Samples of grape leaves and fruits (cortex and flesh) were taken from the orchard after application of both pesticides to determine these residues. The effect of the number of sprays, and the time after the spray application on the residues of both pesticides was studied. The effect of washing the previously treated fruits with both pesticides on the residues of the two pesticides was also investigated. Gas Chromatography / Mass Spectrometry (GC- MS) was used to determine the residues in grapevine organs and in washing water of treated fruit. Results obtained in this study have indicated the presence of both pesticide residues in all tested samples, but chlorpyrifos residues were detected in larger quantities than penconazole residues. Amounts of residues of both pesticides determined in the fruit cortex were larger than that in the fruit flesh. Their residues in washing water of treated fruits were always lower than that in both cortex and flesh. This indicates the systemic action of both pesticides and therefore the process of washing treated fruit with water two weeks after application of the last spray was not efficient in removing the residues in fruit.

Overall results indicated that the determined quantities of chlorpyrifos and penconazole residues in the cortical tissues of treated fruit after application of the 6th sprays of both pesticides were higher than the quantities determined by other authors but they were less than the maximum residue limits (MRLs)

defined by the residue legislation in the other countries. This will lead us to compensate the increase in the pesticide usage by using other non-chemical practices such as integrated pest management (IPM).

Chapter I

Introduction

1. Background

Environmental contaminants, especially pesticide residues in food and water, are now having serious effect on our health and reproduction. These chemicals are marketed in over 40,000 combinations along with 80,000 industrial chemicals. Governments around the world set limits for the amount of residue of fungicides, insecticides or herbicides that are legally allowed in food. These limits of agrochemicals are commonly referred to as maximum residue limits (MRLs) (Jean *et al.*, 2002).

There are high residue levels due to the intensive use of pesticides. Most farmers do not follow the instructions that come with the pesticides. Sometimes they use excessively high rates of application, which increases the residue level. In addition, farmers sometimes do not observe the required safety periods (Mustafa *et al.*, 1993).

Some experts now feel that these residues may be contributing factor and often the leading factors in major diseases that occur because of our endocrine (including immune and nervous) systems are not functioning in harmonic balance.

1.1 Status of grapevine production in Palestine

Agriculture is the backbone of the Palestinian economy, contributing 33% and 24% of the Gross National Products in the West Bank and Gaza strip, respectively (ARIJ 1994). Grapevines are considered the second fruit crop in Palestine after olive; it covers about 9000 hectares (ARIJ, 1994). They are concentrated in the southern part of the West Bank: in Hebron, Bethlehem, Ramallah and Jericho.

Grapevine yard areas have not increased significantly over time. Hebron ranks first with 58.8% of the total vineyard area, followed by Ramallah and

Bethlehem with 26.7%, the Gaza Strip with 7.8%, and finally the northern West Bank (Jenin, Nablus, Tulkarem) with 6.7% (ARIJ 1994) (Table 1).

Table 1.1 Area (in dunums) cultivated with grape in Palestine (ARIJ 1994).

District	1988-89	1989-90	1990-91	1991-92	Average area (dunum)
Hebron	51,303	51,467	51,523	51,700	51,498
Ramallah & Bethlehem	24,200	24,200	22,528	22,600	23,382
Jenin	3,720	3,730	3,730	3,730	3,728
Nablus	1,087	1,117	1,117	1,120	1,110
Tulkarem	956	946	946	946	949
Gaza	6,870	6,870	6,870	6,870	6,870
Total	88,136	88,330	86,714	86,966	87,537

Grapes are temperate climatic plants characterized by climbing stems and prostrate canes. Tendrils fix the canes to any support, aiding in cane distribution and penetration of sunlight. The average production of grapes in Palestine for the last four years is about 52.2 thousand tons, of which Hebron contributed 57.7% to the total (ARIJ, 1994).

1.2 Varieties of cultivated grapes

There are over 13 seeded varieties of grapes grown under rain fed conditions in Palestine. Some of them are consumed as table fruit or after processing, in such forms as Dibis (molasses), jams, Malben (fruit roll), raisins, juice, vinegar, and wine. The most important varieties are: White Grape varieties: such as Dabouki, Zaini, Marrawi, Hamadani, Beiruti (Romani) and Jandali. Red Grape varieties: such as Halawani. Black Grape varieties: such as Shami, Shoyoukhi (Darawishi), Beituni (Baloti), Fahaissi, Motartash. Other varieties of seedless white grape varieties are recently introduced in Jericho and mainly cultivated under irrigation include Berlait and Superior (ARIJ 1994).

1.3 Pests and diseases attack grapevines

There are a lot of pests and diseases which attack grapevines causing severe of damage to the product.

1.3.1 Pests

1.3.1.1 Annual weeds:

Weeds can be controlled by spraying Symnix-50 at a rate of 250-300g / dunum or Symazin-50 at a rate of 250-300g / dunum. Chemical application should be carried out in December directly after the first plauging.

1.3.1.2. Insects

1.3.1.2.1. Phylloxera (*Phylloxera vitifolia*)

Phylloxera attacks the leaves and roots causing galls and knobs on the attacked organs. Severe infection results in the death of infected vines. To control phylloxera, resistant root stocks and infection-free seedlings should be used (Popenoe *et al.*, 1990).

1.3.1.2.2. Grape thrips (*Retithrips syriacus*)

Grape thrips suck the leaves, causing a glazed appearance. They may be controlled using Dursban at a rate of 3cm³ / liter (Popenoe *et al.*, 1990).

1.3.1.2.2. Grape berry moth (*Endopize viteana Clemens*)

Usually found when grapes are wormy at harvest time. It is a dark - colored caterpillar. It passes the winter in the pupal stage on fallen leaves. It emerges about the time the grapes are blooming. There are two broods. The first eats the stems and external portion of the young berries. The second brood lives entirely in the berries. They attack all cultivars. They are most injurious to those cultivars that have compacted clusters since they tend to be protected in such clusters and can feed readily on more than one berry. Some of the injured

grapes fall from the clusters; the rest must be discarded at harvest time. Spraying is the best control measure, but deep cultivation of the leaves in late fall or early spring also aids in control. For few vines, it often pays to pick and destroy the berries infested by the spring brood and to rake and burn the leaves in the fall to reduce the over wintering population. They may be controlled using Dursban at a rate of 2 cm³/ liter or Cymbush at a rate of 2 cm³ / liter (Popenoe *et al.*, 1990).

1.3.2. Diseases

1.3.2.1. Powdery mildew (*Uncinula necator*)

Powdery mildew is an important disease of grapes. This disease affects the leaves, flowers, and fruits (Figures 1.1 and 1.2). It is most likely to attack the leaves and fruit during the spring and fall when the weather is warm and the humidity is not high. Most powdery mildews develop as thin layers of mycelium on plant's surface. Conidia or resting bodies make up the bulk and are the primary means of dispersal (Figure 1.3). Powdery mildew conidia are carried by the wind and rain to new hosts. Excess water on the plant's surface can kill conidia and inhibit growth of mycelia, and both conidia and mycelia are sensitive to extreme heat and direct sunlight (Grove and Watson, 1997).



Figure 1.1 Grape leaf severely infected with powdery mildew (Grove and Watson, 1997)



Figure1. 2 Grape fruit cluster infected with powdery mildew (Grove and Watson, 1997)

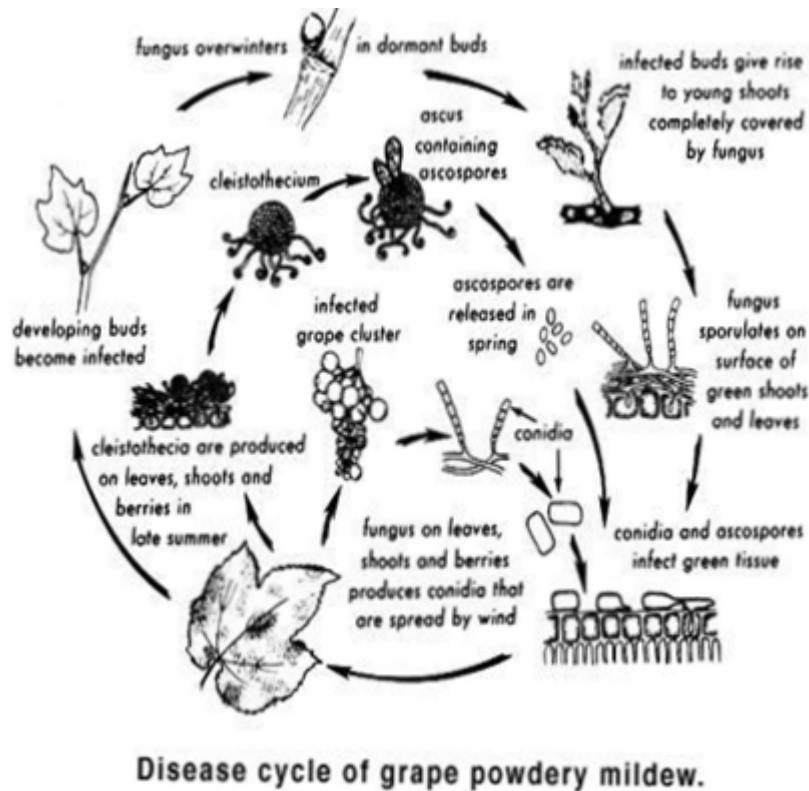


Figure 1.3 Disease cycle of grape powdery mildew (Grove and Watson, 1997).

To reduce the chance of the disease incidence, grape vines should be planted in non-shaded areas. Spacing between plants provides enough aeration and growing room. Pruning and thinning out branches and monitoring for any signs of infection, Collecting infected leaves and fruit are recommended and providing enough moisture, by watering in the morning or late afternoon may reduce the disease severity (Grove and Watson, 1997).

1.4 Status of pesticides in Palestine

West Bank agriculture has, in the last few years, increased in sophistication, and this has had many negative side effects, of which the overuse of pesticides could prove to be the most serious problem facing the Palestinian agriculture (WRI, 1994; Igbedioh, 1991).

A warm climate combined with the prominence of agriculture in the Palestinian economy help to make pesticide usage widespread. A total of 123 pesticides are currently being used in the West Bank. Among them, fourteen pesticides are internationally suspended, cancelled or banned. Seven of these pesticides are members of the "dirty dozen" (ARIJ, 1995).

The total cultivated area of the West Bank is around 2 million dunums. Of this, only one hundred thousand dunums are under irrigation, while 1.6 million dunums are rain fed and 300 thousand dunums are fallow lands (ARIJ 1994). It is estimated that 96.6% of irrigated land and 87.0% of rain fed land is treated with pesticides. The average seasonal consumption of pesticides was found to be around 4 kg/dunum in open irrigated fields and 6.5 kg/dunum under plastic (ARIJ, 1995).

Until recently, pesticides were not considered a problem in the West Bank. On the contrary, their use was considered a sign of progress and modernization. Pesticides are seen as a cure-all, without consideration for health or the environment. With this attitude prevalent among the agriculture, farmer's use of pesticides increased, particularly in irrigated farming. Unfortunately, this increase has been accompanied by a full understanding of the impacts of pesticides on human health, beneficial organisms and the environment (Sansour, 1992; Igbedioh, 1991). But the lack of mechanisms, institutions and laws which control and monitor the sale and proper application of pesticides has left pesticide use in Palestine virtually unrelated. In general, farmers in Palestine are unaware of the risks associated with the use of agrochemicals of all kinds of pesticides: insecticides, herbicides, fungicides, hormones, and rodenticides, and their source of information is limited to their own experience, word of mouth, extension agents and pesticide-selling agents (ARIJ, 1995). Thus, the safe and effective uses of pesticides in Palestine face serious problems. Also the excessive using of pesticides leads to insect

resistance, high residues level in fruits, environmental pollution, and increasing the costs of production (Mustafa, 1991).

1.5 Pesticides used in this study

Penconazole (Ofir®), and chlorpyrifos (Dursban®) were among the most frequently used pesticides in Palestine to control powdery mildew and fruit moth larva of grape. Therefore, in the present work, the above mentioned pesticides have been studied.

1.5.1 Penconazole (Ofir®)

Penconazole is a systemic triazole fungicide with preventive and curative properties for the control of powdery mildew disease of different crops. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes. It is used on fruit, especially apples and grapes, and vegetables (Tokelaar and Kotten- Vermeulen, 1992).

The Physical Properties of penconazole are:

Trade and Other Names: CCA-71818, Topas, Ofir, Topaz, Omnex, Award.

Chemical Name: 1-[2-(2, 4-dichlorophenyl) pentyl]-1H-1,2,4-triazole

Molecular Formula: $C_{13}H_{15}Cl_2N_3$

Molecular Weight: 284.2

Activity: Fungicide (conazole fungicide)

Structure:

Formulations: Emulsifiable concentrate, wettable powder.

Solubility: Solubility in water 70 ppm at (20°C), Acetone 700 g/kg, Methanol 800 g/kg, Dimethylbenzene 500 g/L. Stable below 350°C and stable to hydrolyze.

Melting point: 60°C.

Vapor pressure: 0.21mPa (20 ppm).

Toxicity: Acute oral LD50 for rats is 2125mg/kg, acute skin LD50 for rats is >3000mg/kg, slight irritation to the eye and skin of rabbits and with very low toxicity for bees.

1.5.2 Chlorpyrifos (Dursban®)

Chlorpyrifos is a broad-spectrum organophosphate insecticide. While originally used primarily to kill mosquitoes, it is no longer registered for this use. Chlorpyrifos is effective in controlling cutworms such as, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is used as an insecticide on grain, cotton, fruit, nut and vegetable crops, and as well as on lawns and ornamental plants. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings, farm buildings, storage bins, and commercial establishments. Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison. Chlorpyrifos is one of the most widely used insecticides in the United States. It is registered for use in more than 800 products. According to Environmental Protection Agency (EPA), about half of the estimated 20 million pounds applied annually is used on 40 different agricultural crops. Chlorpyrifos is among a family of 45 pesticides known as organophosphates that attack the nervous system and are under review by the (EPA) because of their potential health effects on children (Farooqui, 2000).

Physical properties of chlorpyrifos:

Trade and other names: Trade names include Dursban, Brodan, Detmol UA, Dowco 179, Dursban, Empire, Eradex, Lorsban, Paqeant, Piridane, Scout, and Stipend.

Toxicity: It is highly toxic insecticide. Products containing chlorpyrifos bear the Signal Word WARNING or CAUTION, depending on the toxicity of the formulation. It is classified as a General Use Pesticide (GUP). The oral LD50 for chlorpyrifos in rats is 95 to 270 mg/kg (Gallo and Lawryk, 1991; Kidd and James, 1991); 1000 mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep (Gallo and Lawryk, 1991; Kidd and James, 1991, Gosseline *et al.*, 1984).

Chemical Class: organophosphate

Molecular Formula: C₉H₁₁Cl₃NO₃PS

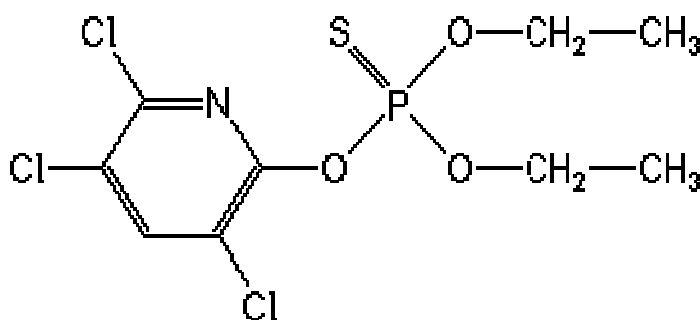
Molecular Weight: 350.62

Formulation: It is available as granules, wettable powder, dustable powder, and emulsifiable concentrate.

Solubility: solubility in water is 2mg/L at 25°C, readily soluble in acetone, chloroform, carbon disulfide, diethyl ether, xylene, methylene chloride and methanol (Kidd and James, 1991).

Chemical Name: O,O-diethyl 1-O-(3,5,6-trichloro-2-pyridyl) phosphorothionate (Kidd and James, 1991).

Structure:



Melting Point: 41.5-44 °C (Kidd and James, 1991).

Vapor Pressure: 2.5 mPa at 25 °C (Kidd and James, 1991).

Environmental Fate:

- **Breakdown in soil and groundwater:** Chlorpyrifos is moderately persistent in soils. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions (Howard, 1991; Waushope *et al.*, 1992). Chlorpyrifos was less persistent in the soils with a higher pH (Racke, 1992). Soil half-life was not affected by soil texture or organic matter content. In anaerobic soils, the half-life was 15 days in loamy soil and 58 days in clay soil (U.S. E.P.A, 1989). Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. When applied to moist soils, the volatility half-life of chlorpyrifos was 45 to 163 hours, with 62 to 89% of the applied chlorpyrifos remaining on the soil after 36 hours (Racke, 1992). In another study, 2.6 and 9.3% of the chlorpyrifos applied to sand or silt loamy soil remained after 30 days (Racke, 1992). Chlorpyrifos could be adsorbed strongly to soil particles and it is not readily soluble in water (Waushope *et al.*, 1992; Racke, 1992). It is therefore immobile in soils and unlikely to leach or to contaminate groundwater (Racke, 1992).
- **Breakdown in water:** The concentration and persistence of chlorpyrifos in water will vary depending on the type of formulation. Volatilization is probably the primary route of loss of chlorpyrifos from water. Chlorpyrifos is unstable in water, at pH 7.0 and 25 °C; it had a half-life of 35 to 78 days (Howard, 1991).
- **Breakdown in vegetation:** Chlorpyrifos may be toxic to some plants, such as lettuce (McEwen and Stephenson, 1979). Residues remain on plant surfaces for approximately 10 to 14 days. Data indicate that this insecticide and its soil metabolites can accumulate in certain crops (U.S.P.H.S., 1995).

Ecological Effects:

Chlorpyrifos is moderately to very highly toxic to birds; it is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms (U.S. E.P.A, 1989). Also uses of chlorpyrifos pose a serious hazard to wildlife and honeybees (Kidd and James, 1991; U.S. E.P.A, 1984).

1.5.3 Quantitative determination of Chlorpyrifos (Dursban®) and Penconazol (Ofir®)

Many reviews and methods have been published on the quantitative determination of penconazol (Ofir®) and chlorpyrifos (dursban®). Oliva *et al.*, 1999 studied a rapid gas chromatographic method for determination of residue levels of chlorpyrifos insecticide and penconazol fungicide in grapes. An on-line microextraction method was used. The matrix of acetone- dichloromethane (1:1v/v) was filtered and concentrated. Electron-capture detection for chlorpyrifos and penconazol was utilized. No clean up was necessary because there were no interference in the area of interest of the chromatogram. Linearity in the range 0.02-2 ppm was checked. In all cases, the correlation coefficient was 0.997. Recoveries from spiked grapes ranged from 78% to 101 %. Limits of determination in grape were 0.00235, 0.00229 and 0.00229 ppm for chlorpyrifos and 0.00352, 0.00359 and 0.00340 ppm for penconazole.

Navarro *et al.*, 2002 determined the pesticides in grape berries harvested at two hours, 1, 3, 7, 14 and 28 days after phytosanitary treatment. The determination of residues was carried out by GC-ECD for chlorpyrifos and penconazole. The residue levels detected in the study immediately after pesticide application were 6.91 and 0.14 ppm, for chlorpyrifos and penconazole respectively, but these levels fell to 0.14, 0.03, ppm after 28 days of the application for chlorpyrifos and penconazole respectively. The calculated half-

life times were 4.4 and 6.6 days for both pesticides respectively. In the case of chlorpyrifos, the time necessary to reach the concentration of the corresponding maximum residue limit (MRL) was below their designated days to harvest times. The theoretical initial residue levels of penconazole (0.12 ppm) were below the maximum residue limit (MRL) established for *Vitis vinifera* by Spanish legislation.

The influence of wine- making processes on the disappearance of chlorpyrifos and penconazole in red wines elaborated through carbonic maceration has been studied by Garcia *et al.*, 1999. The vineyard was treated with the pesticide three hours before the grapes were harvest. Under laboratory conditions, the grapes (10 kg) were introduced into recipients of adequate capacity in carbonic an aerobiosis. Three replications were made in each case. From this moment until the finished wine, several samples of grapes, must (free- run and press juice), wine, pomace and lees were taken to study the disappearance of the pesticide residues. The initial levels of residues in grapes oscillated between 0.28 ppm for penconazole and 1 ppm for chlorpyrifos. Ten days after the beginning of maceration, the compound that remains in the highest proportion in grapes was chlorpyrifos (84.9 %). On the contrary, in the free- run juice, the lowest percentage corresponded to chlorpyrifos (0.1%). After pressing the grape, the percentages eliminated in pomace with regard to initial values (82.7 %) for chlorpyrifos and 1.8 % in the press juice. In finished wine residues were present only in penconazole.

Fernandez *et al.*, 2003, presented an analytical method for the determination of residues of 10 fungicides (penconazole one of them) in white grape for vinification. It is based on organic solvent extraction with dichloromethane-acetone (75:25, v/v) followed by gas chromatography with mass spectrometric detection. The applicability of the method was evaluated by analysis of 5 different white grapes produced in the Rias Baixas area in Galicia

(northwestern Spain) for vinification. Results showed that concentrations of the fungicides identified in grapes were lower than the MRLs established by the European legislation.

Liapis *et al.*, 2003 described rapid, selective and sensitive multi-residue method for the determination of six common pesticides in stone fruit samples. The proposed method involves the extraction of the pesticides with the use of acetone solvent followed by liquid-liquid partition with a mixture of dichloromethane and light petroleum (40-60°C) and subsequent determination by a GC-MS system using ion trap technology in negative ion CI mode. The recoveries of chlorpyrifos and parathion methyl examined in the concentration range 0.02-0.2 ppm were 95.5 ± 7.5 to $145 \pm 3.6\%$; the highest mean recovery (145%) for chlorpyrifos is attributed to a matrix enhancement effect. The limits of quantification in apricots were 0.01 ppm for chlorpyrifos. The method was applied successfully to the determination of the target pesticides in 32 samples of stone fruits (apricots and peaches).

Correia *et al.*, 2001 developed a method using SPME and GC-ECD (gas chromatography electron capture detector) for the determination of some pesticide residues in grape samples. The procedure only needs dilution as sample pretreatment and is therefore simple, fast and solvent-free. Fungicide (penconazole) and insecticide (chlorpyrifos) can be quantified. Good linearity was observed for the two compounds in the range 5-100 µg/L. The reproducibility for the measurements was found acceptable (with residues (RSD) <20%). Detection limits of 11µg/L, on average, are sufficiently below the proposed maximum residue limits for these compounds in wine. The analytical method was applied to the determination of these compounds in Portuguese grape samples from the Demarcated Region of Alentejo, Portugal.

Zambonin *et al.*, 2002 developed a SPME-GC-MS method for the determination of triazole residues, such as penconazole. The method has been

successfully applied to the analysis of strawberries and wine samples. The procedure is solvent-free, simple and highly sensitive. Within-day and day-to-day residues ranged between 2-11% and 7-28%, respectively. Since the detection limits achieved by this method are well below the maximum residue levels for wine (or grapes) and strawberries recommended by the European legislation, it can be conveniently used as a low-cost rapid screening method for the contamination of the considered samples.

Song *et al.*, 2002 have investigated a novel green method using flow injection chemiluminescence's with controlled-reagent-release technology for the rapid and sensitivity monitoring of sub-nanogram amounts of chlorpyrifos. The analytical reagents involved in the chemiluminescence (CL) reaction, luminol and periodate, were both immobilized on an anion-exchange column. The CL signals produced by the reaction between luminol and periodate, which were eluted from the column through water injection, were decreased in the presence of chlorpyrifos. The decrease of CL intensity was linear over the logarithm of concentration of chlorpyrifos ranging from 0.48 to 484 ppm and the limit of detection was 0.18ppm .At a flow rate of 2 ml/min, the determination of chlorpyrifos, including sampling and washing, could be performed in 0.5 min with a residue of < 3%. The proposed method was applied successfully in an assay of remnant chlorpyrifos on fruits such as orange and shaddock with the recovery of 94.4 - 107.4%. The change of the concentration of chlorpyrifos in a water sample was also investigated, and the variation rate was 99.96% during 35 hours in the open air.

Mustafa *et al.*, 1993 analyzed dimethoate and its metabolite omethoate residues in cucumber fruits using gas liquid chromatography (GLC) with a nitrogen phosphorus detector (NPD). Different treatments were used to reduce the residues of dimethoate from cucumber fruits sprayed with dimethoate 25 ml/ 20 liter solution. The different removal treatments were evaluated after 1

hour, 2 days, 4 days, and 6 days of treating cucumber fruits with dimethoate. These treatments included peeling, dipping in tap water with soap for 2 minutes, dipping cucumber for 10 minutes, hand rubbing, washing cucumber fruit under tap water for few seconds. The result showed that the percentages of removal were 52 % for peeling, 34% for dipping in tap water with soap for 2 minutes, 19% for dipping for 10 minutes, 18.6% for hand rubbing and 11.2% for washing cucumber under tap water.

1.5.4 Objectives of the research

This research aimed at studying the following:

1- Determination of the residual quantities of the most widely used pesticides chlorpyrifos (Dursban®) and penconazole (Ofir®) in grape vineyards in Palestine against grape powdery mildew and grapeberry moth larva (disease and pest attack grapevines).

2- Comparison of the determined residues of both pesticides by gas chromatography with the maximum residue limits (MRLs) defined by the international legislation.

Chapter II

Materials and Methods

All chemicals and solvents used in the present work are of analytical grade obtained from Merck and Sigma.

2.1 Pesticide standard solutions used in the study

2.1.1 Chlorpyrifos standard solution

A 1000 ppm standard solution of chlorpyrifos was prepared by transferring exactly 2.08 ml of (480 g/L) solution of chlorpyrifos (Dow Agro Sciences, Israeli) into a 1- liter volumetric flask. The volume was completed to the mark with water. Five ml of the latter solution was transferred into a 100 ml reparatory funnel, mixed with 25 ml ethyl acetate and then shacked for two minutes. The organic layer formed after shaking was separated and then transferred into a 50 ml volumetric flask. The volume was completed to the mark using ethyl acetate. About 2 g of anhydrous sodium sulfate was then added to the solution then shacked for few minutes in order to remove the traces of water remained in the solution. Two ml of the prepared standard solution (100 ppm) were transferred into the gas chromatography vials for injection.

2.1.2 Penconazol standard solution

To prepare 1000 ppm standard solution of penconazole, the same procedure that was used for chlorpyrifos was followed. Except that 5 ml of the (200 g/L) solution of penconazole (Novartis, Israeli) instead of 2.08 ml for chlorpyrifos standard solution were taken during preparation of penconazole standard solution.

2.2 Equipment

2.2.1 Field equipments

Sprayer with plastic drum (20 Liter capacity), in addition to protective clothes were used in the spraying.

2.2.2 Laboratory equipments

Blender: (model 34B147 (800), 240 volts AC, 50- 60 HZ, 1.5 AMPS, U.S. PAT. NO' S, NEW HARTFORD, Connecticut 06057) with a speed of 20000 rounds per minute (rpm).

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

Operation condition: injector 250°C, GC/MS interface 275°C, helium carrier gas at a flow rate of 6.2 ml/min at 25°C, splitless injection mode.

Temperature program: 60°C for 3 min, rose at 10°C/ min to 300°C then held for 5 min at 300°C.

Retention time for chlorpyrifos is 52.336 min, and for penconazole is 55.084 min as shown in figure (3.1 and 3.2).

2.3 Field experiments

2.3.1 Grapevine orchard used

An orchard of grapevine located at Bit Eiba village near Nablus city was used in this study. This orchard has moderate climates during grapevine growing season (average seasonal temperature was 26 °C and average relative humidity was 55 %) and good water – holding capacity for the soil in which the grapevines are planted.

2.3.2 Sampling procedure

Grapevines (variety Zeini) that were characterized by medium-sized berries with juicy fruit suitable for consumption and processing were used in the

present work. Protective sprays with chlorpyrifos (Dursban®) and penconazole (Ofir®) were applied to protect the vines from powdery mildew infection and grape fruit moth larvae attack during growing season. Each grapevine in the orchard was treated every 2 weeks with penconazole (Ofir®) pesticide (50 mg/L of spray solution) starting at the beginning of growing season (unfolding of the leaves from their buds). During the early fruit ripening, Ofir® was mixed with Dursban® (50 mg/ L penconazole and 0.96 mg/ L chlorpyrifos then sprayed every 2 weeks. Fruit and leaf samples were picked up at four intervals: 14 days after the 5th spray, 1,9,14 days after the 6th spray. The samples were stored 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 solutions were stored in the refrigerator at 2-4°C for analysis by gas chromatography /mass spectrometer. Each sample was represented by 3 replicates used for calculation of the mean value of pesticide residue level for both pesticides.

2.4 Extraction Procedure

2.4.1 Extraction of chlorpyrifos and penconazole from leaves, flesh and cortex of grape berries

The same extraction procedure was followed for both penconazol (Ofir®) and chlorpyrifos (Dursban®). Fifty gram samples of leaves or fruits were blended for 3 minutes with 50g of anhydrous sodium sulphate and 100ml ethyl acetate. The solution was filtered through Buchner Funnel. Finally, the solution was evaporated to dryness on water bath (70°C), then the residues were diluted with 2 ml of ethyl acetate and transferred into a 2 ml vial stored at -30°C until analysis by gas chromatography/ mass spectrometer (GC/ MS) in the selected ion monitoring mode.

2.4.2 Extraction of chlorpyrifos and penconazole from washing water solution of treated berries

About 500 grams of berries were soaked in about 200 ml of water for 5 minutes. Washing water solution was transferred into a 500-ml separatory funnel, and 100 ml of ethyl acetate were added. The two liquids were shaken for 2 minutes. The organic layer was separated from the mixture; about 2 g of anhydrous sodium sulphate were added to the organic layer, then shaken 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 on water bath (70 °C). Then the residue was diluted with 2 ml of ethyl acetate and transferred into a 2-ml vial. This extracted sample was stored at -30°C until injection into a gas chromatography / mass spectrometer.

2.4.3. Gas chromatographic / mass spectrometric analysis

The concentrates containing penconazole and chlorpyrifos were analyzed using gas chromatography / mass spectrometry in the selected ion monitoring mode. The obtained results were compared with the results obtained for standards of penconazole and chlorpyrifos analyzed under the same conditions (Figs. 3.1 and 3.2). Chlorpyrifos and penconazole residues in each sample were calculated using the following formula:

$$C_s \text{ (ppm)} = (A_s \cdot F_v) \div A_{st} \div W_t \cdot C_{st}$$

$$= (A_s / A_{st}) (F_v / W_t) C_{st}$$

Where,

C_s = Concentration of residues in sample in ppm.

C_{st} = Concentration of residues in standard solution in ppm

A_s = Peak area obtained for the sample.

A_{st} = Peak area obtained for standard solution.

W_t = Weight of analysis sample in grams.

F_v = Final volume of the analyzed solution in ml.

Chapter III

Results

3.1 Retention time of penconazole and chlorpyrifos

By applying the recommended gas chromatography / mass spectrophotometer operation conditions, standard solutions of 100 ppm penconazole (Ofir ®) and 100 ppm chlorpyrifos were analyzed. The obtained results are presented in figures (3.1 and 3.2). These figures indicate that the retention time of penconazole is 52.336 min, while the retention time of chlorpyrifos was 55.084 min.

The selected ion-monitoring mode was used in the present work in order to eliminate the interference from other compounds that may be present in the samples. The same procedure was followed for determination of penconazole and chlorpyrifos residues in the samples analyzed.

Typical GC/MS chromatograms for quantitative determination of penconazole and chlorpyrifos in samples of cortex, leaves and flesh of grape analyzed following the recommend procedure are presented in figures (3.3 a, b and c), respectively.

Figure 3.1 GC/ MS chromatograms of 100 ppm chlorpyrifos standard solution analyzed following the recommended procedure.

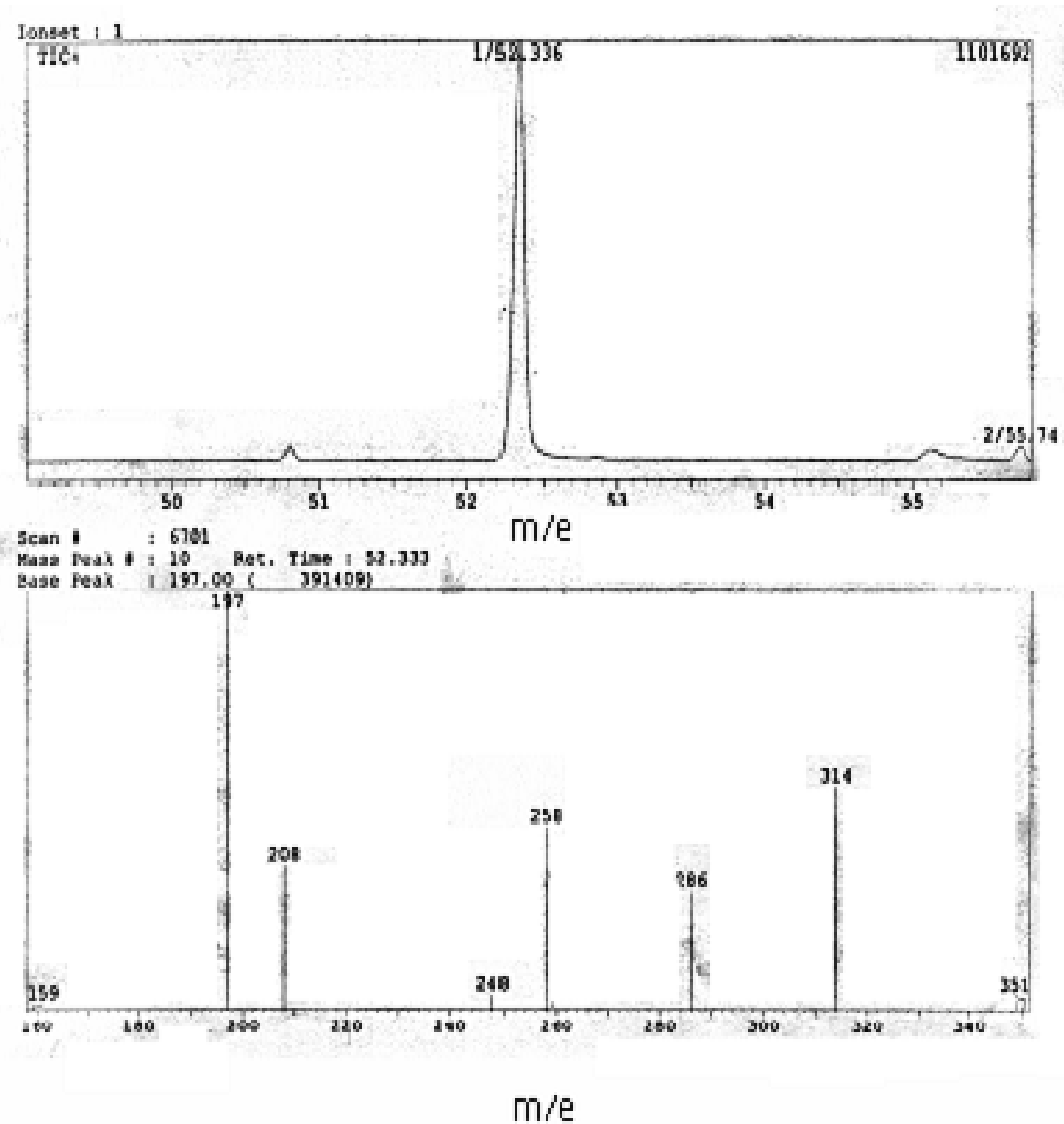


Figure 3.2 GC/ MS chromatograms of 100 ppm penconazole standard solution analyzed following the recommended procedure.

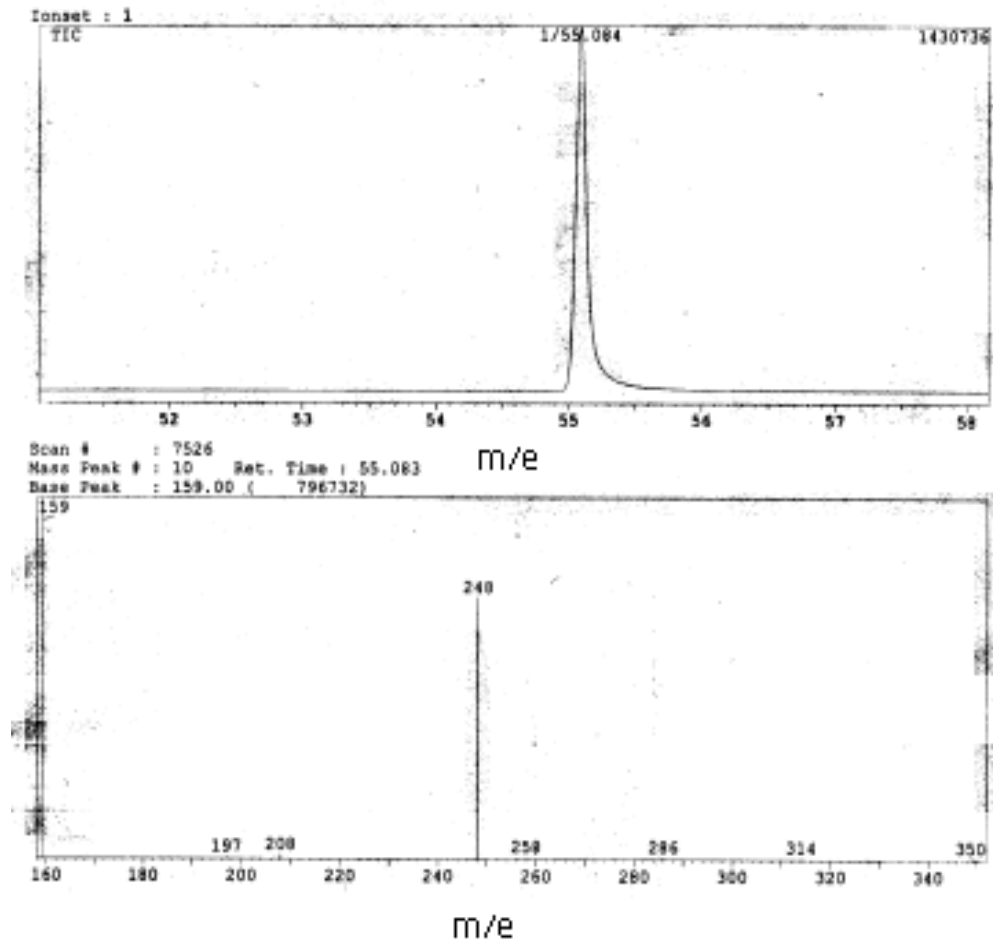


Figure 3.3a Typical GC /MS chromatogram of grape cortex sample analyzed following the recommended procedures.

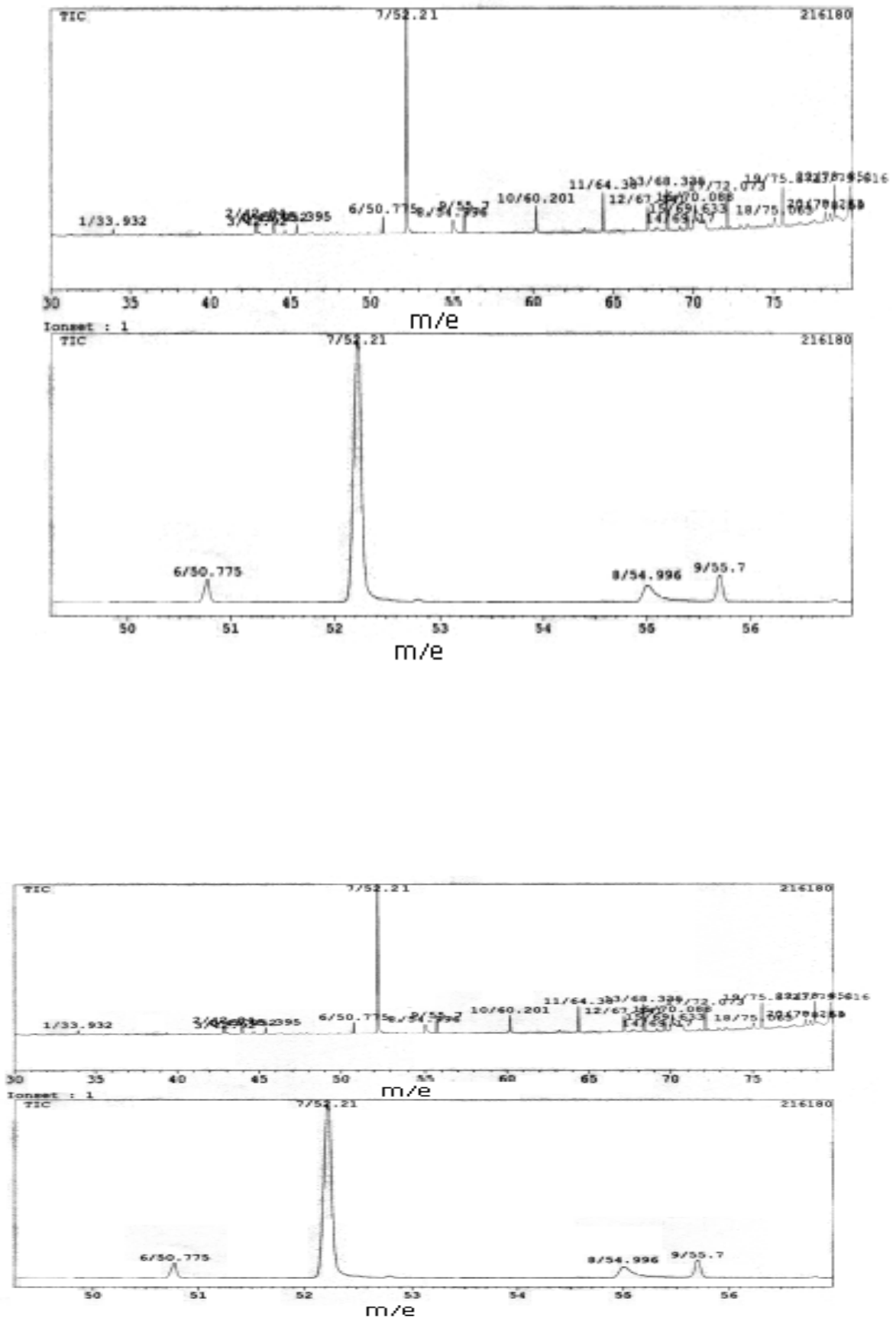
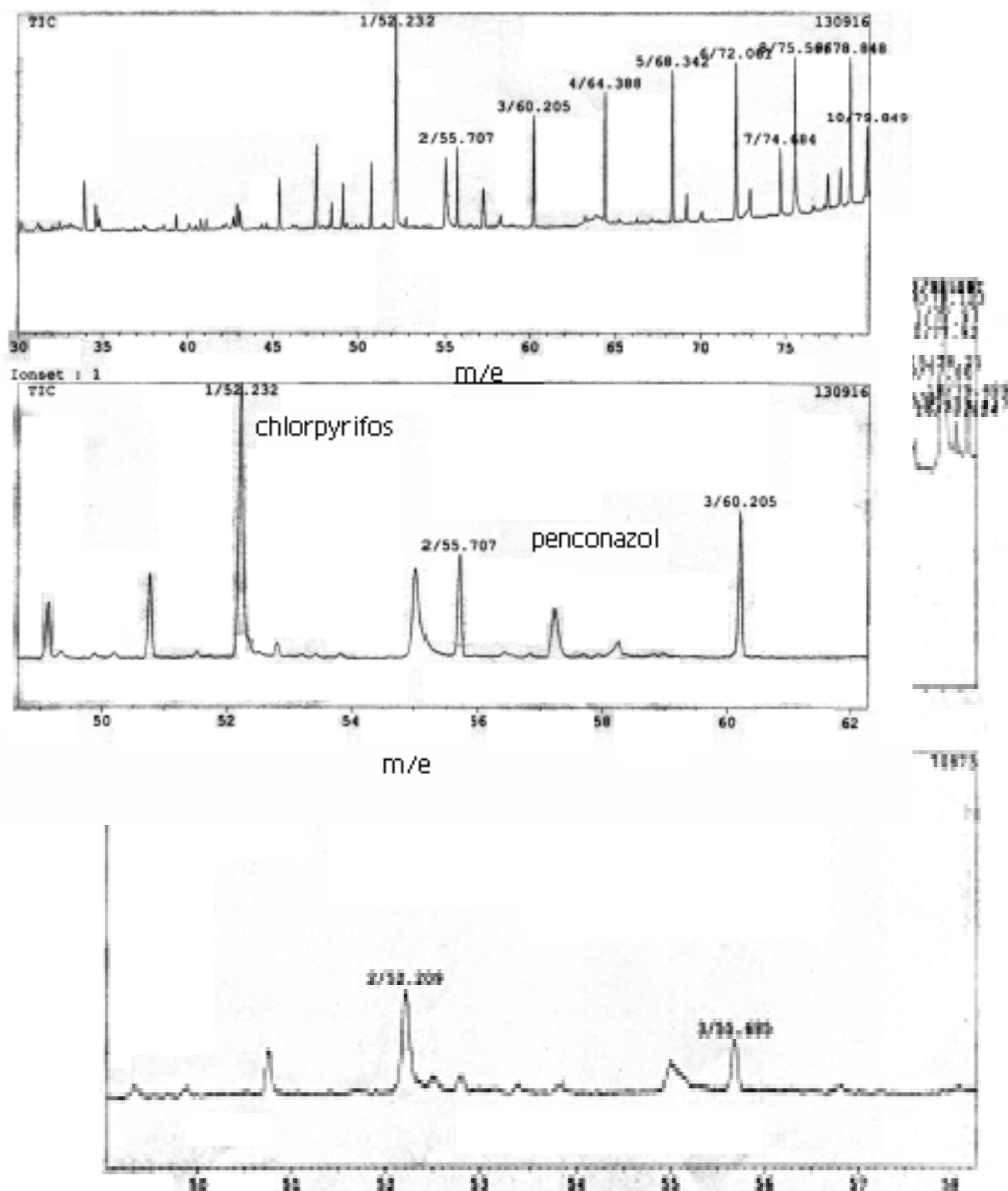


Figure 3.3b Typical GC /MS chromatogram of grape leaves sample analyzed following the recommended procedures.

Figure 3.3c Typical GC /MS chromatogram of grape flesh sample analyzed following the recommended procedures.



3.2 Effect of number of sprays on penconazole residues in grape

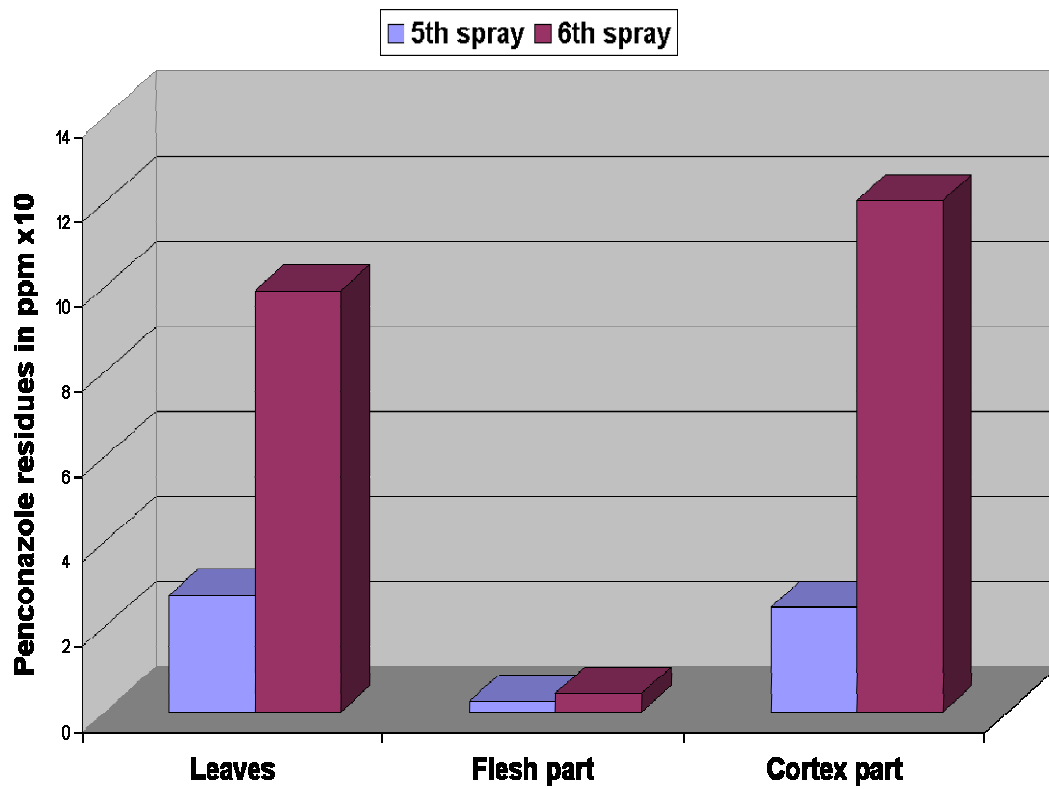
The effect of number of sprays using 50 mg /L penconazole (Ofir ®) solution on the residues of this fungicide in grape leaves, in flesh and cortex of the fruit have been studied. The obtained results (Table 3-1 and Fig. 3-4) indicate that penconazole residues in the three parts of the grapevine after 14 days of spraying increased by increasing the number of sprays (flesh part: samples 6 and 13, cortex: samples 7 and 14, and leaves: samples 8 and 12).

Table 3.1 Penconazole (Ofir®) residues (ppm) in grape leaves and fruit after 6 applications of the fungicide spray on grapevine grown in Beit- Eba, Nablus during the growing season (average seasonal temperature 26°C and relative humidity 55%).

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

* Average of three measurements

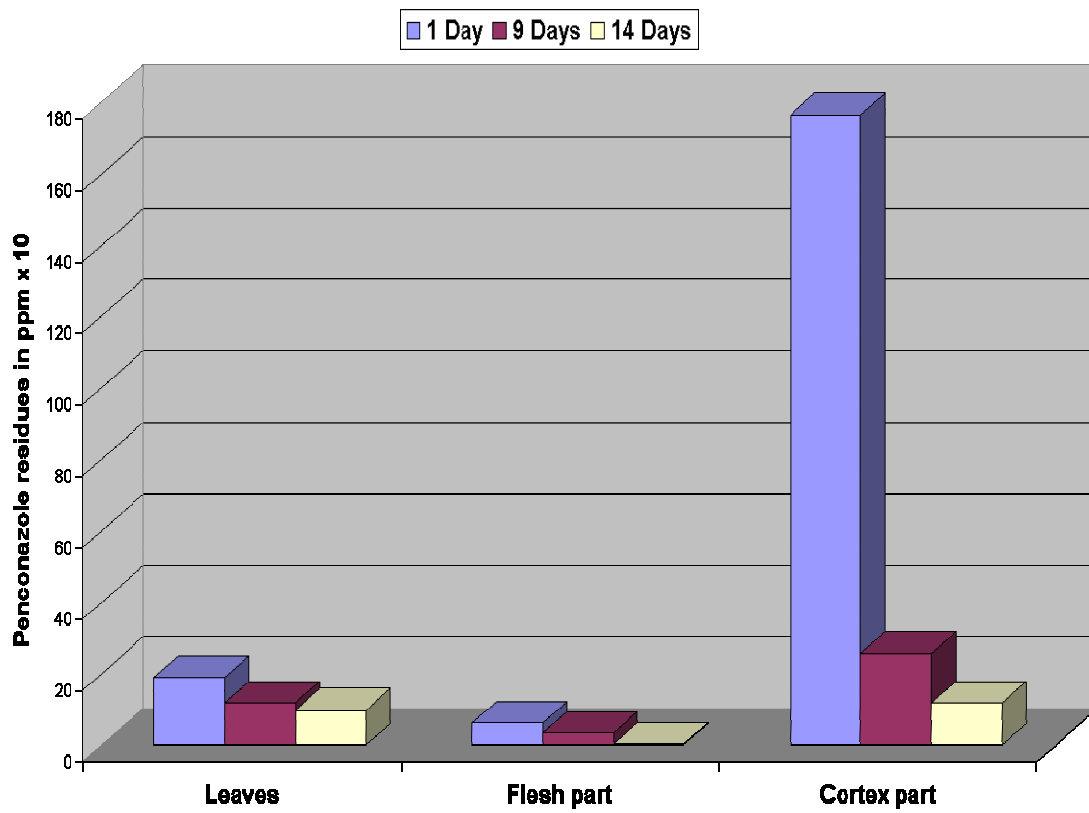
Figure 3.4 Effect of number of sprays on penconazole (Ofir ®) residues in ppm in grape leaves and fruit using 50 ppm penconazole spray solution



3.3 Effect of time after spraying on the Penconazole residues in grape

The effect of time after spraying of grapevine with 50 ppm Penconazole (Ofir ®) solution on the residues of this fungicide in different parts of grapevine has been studied. The obtained results (Table 3.1 and Fig. 3-5) indicated that Penconazole residues in different parts of the grapevine decreased by increasing the time after spraying. (19.09×10^{-3} ppm after 1 day, to 12.04×10^{-3} ppm after 9 days, and to 9.93×10^{-3} ppm after 14 days at the 6th spray on leaves). This is evidence that the Penconazole residues decrease by increasing time after spraying. This decline 14 days after spraying on leaves calculated to be 48 %, while it was 93 % in the cortex and flesh of the fruit.

Figure 3.5 Effect of time (in days) after the 6th spray application on penconazole (Ofir®) residues in grape leaves and fruit (concentration 50 ppm of penconazole solution).



3.4 Effect of number of sprays on chlorpyrifos residues in grape

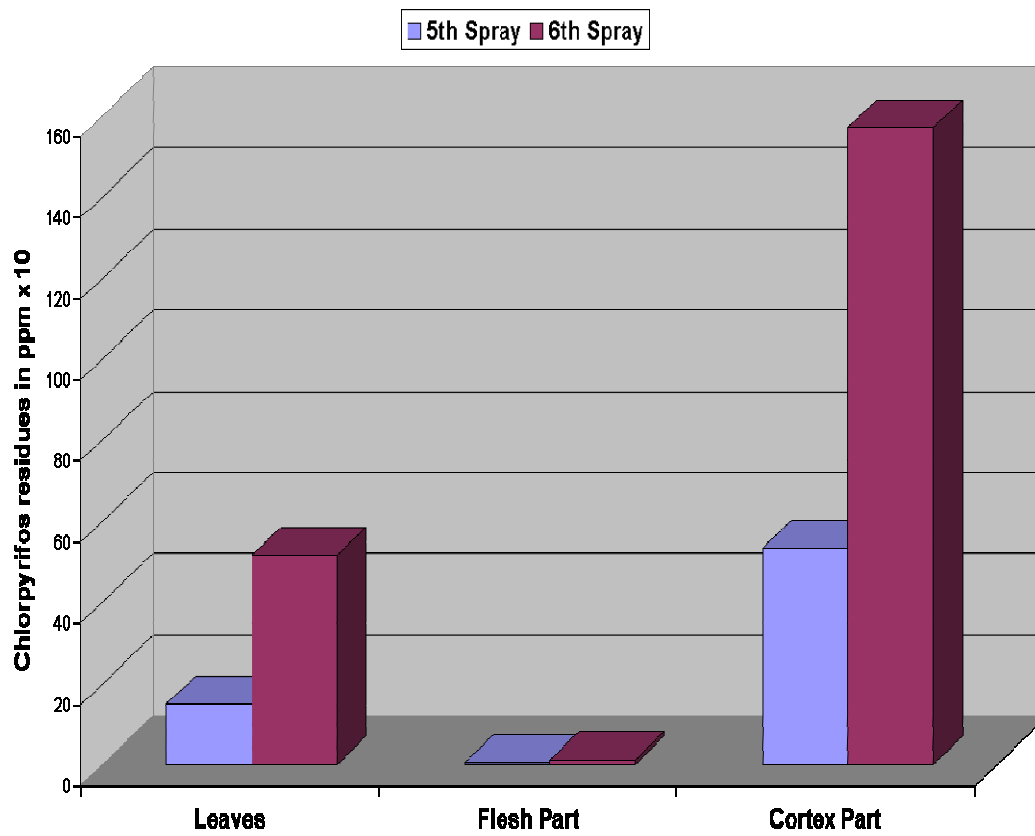
The effect of number of sprays using 0.96 ppm chlorpyrifos (Dursban ®) solution on the residues of this insecticide in leaves, flesh and cortex of the grape have been studied. The obtained results (Table 3.2 and Fig. 3.6) indicate that chlorpyrifos residues in the leaves, flesh and cortex of fruit after 14 days of spraying increased by increasing the number of sprays (flesh part: samples 6 and 13, cortex: samples 7 and 14 and leaves: samples 8 and 12).

Table 3.2 Chlorpyrifos (Dursban®) residues (in ppm) in grape leaf and fruit after 6 applications of the insecticide spray on grapevine grown in Beit-Eba, Nablus during the growing season (average seasonal temperature 26°C and relative humidity 55%).

Sample Number	Applicatio number	Sample type	Time in days after spraying	Chlorpyrifos residues in (X 10 ⁻³ ppm)*
6	5 th	Flesh Part	14	0.39
7	5 th	Cortex	14	53.27
8	5 th	Leaves	14	15.01
1	6 th	Leaves	1	106.47
3	6 th	Flesh part	1	7.14
4	6 th	Cortex	1	196.39
10	6 th	Cortex	9	175.21
11	6 th	Flesh part	9	4.11
16	6 th	Leaves	9	64.03
12	6 th	Leaves	14	51.56
13	6 th	Flesh part	14	1.19
14	6 th	Cortex	14	157.03

* Average of three measurements

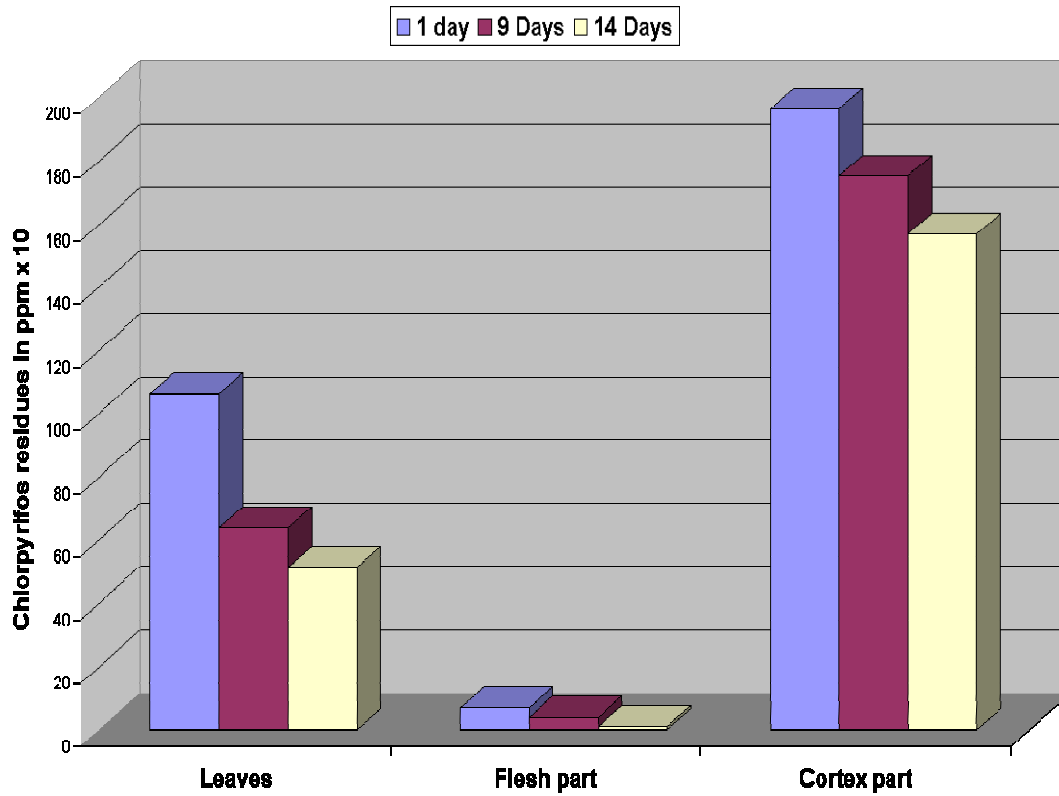
Figure 3.6 Effect of number of sprays on chlorpyrifos residues (in ppm) in grape leaves and fruit (concentration: 0.96 ppm chlorpyrifos solution).



3.5 Effect of time after spraying on the chlorpyrifos residues in grape

The effect of time after spraying of grapevine with 0.96 ppm chlorpyrifos (Dursban ®) solution on the residues of this insecticide in different parts of grapevine has been studied. The obtained results (Table 3-2 and Fig. 3-7) indicate that chlorpyrifos residues in different parts of the grapevine decreased by increasing the time after spraying (106.47×10^{-3} ppm after 1 day to 64.03×10^{-3} ppm after 9 days and to 51.56×10^{-3} ppm after 14 days at the 6th spray on leaves). This is 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.

Figure 3.7 Effect of time (in days) after the 6th spray application on chlorpyrifos (Dursban ®) residues in grape leaves and fruit (concentration: 0.96 ppm of chlorpyrifos solution).



3.6 Determination of penconazole and chlorpyrifos residues in washing water solution

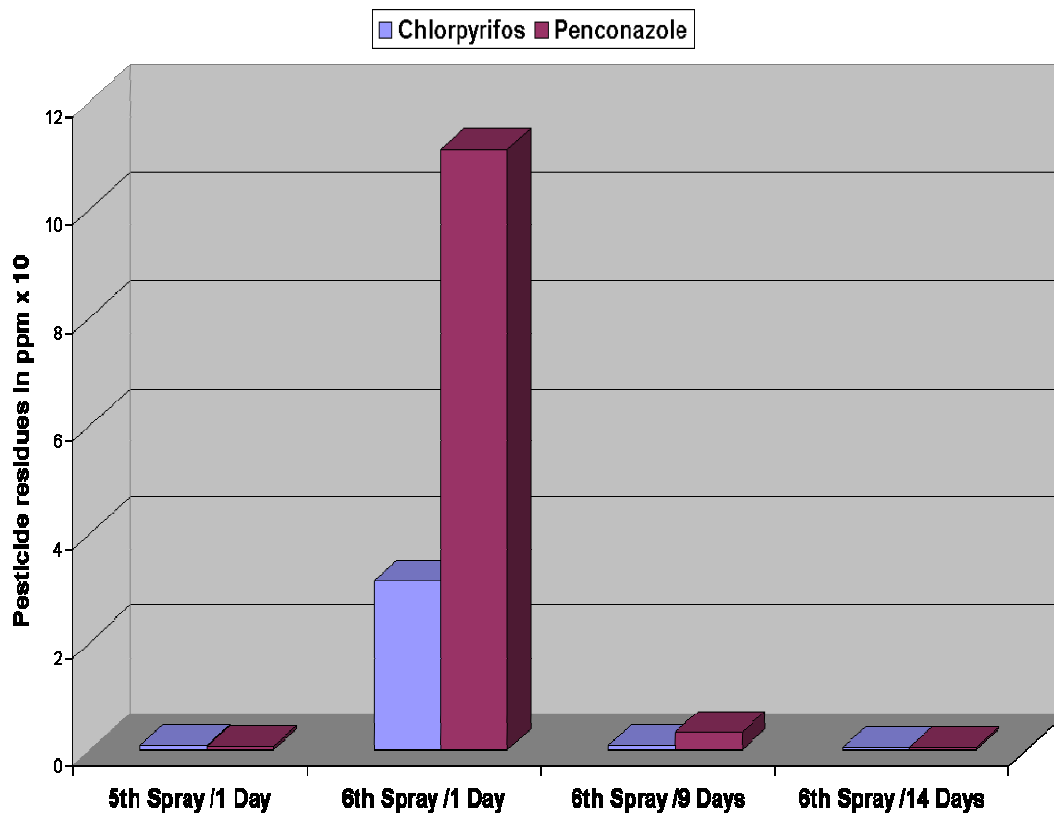
The residues of these pesticides in washing solution that resulted from washing sprayed grape berries with water have been studied. The obtained results (Table 3-3 and Fig 3-8) indicate that the concentration of penconazole and chlorpyrifos in the washing solution did not increase by increasing the number of sprays. On the other hand, the concentrations of both pesticides decreased by increasing the time after spraying. This is evidence that the penconazole and chlorpyrifos molecules were absorbed by the fruit while small quantities of both pesticides can be washed by water. The decrease in the concentration of both pesticides on the fruit surface could be attributed to two factors. The systemic action of both pesticides that will be exhibited after being absorbed by treated cortical tissues of grape berries, and the degradation of these pesticides due to the effect of environmental conditions.

Table 3.3 Determination of penconazole (Ofir®) and chlorpyrifos (Dursban®) residues in washing water solution of treated berries.

Sample Number	Application number	Time in days after spraying	Penconazol residue (X 10 ⁻³ ppm)*	Chlorpyrifos residue (X 10 ⁻³ ppm)*
5	5 th	14	0.06	0.08
2	6 th	1	3.11	11.11
9	6 th	9	0.08	0.33
15	6 th	14	0,04	0.05

* Average of three measurements

Figure 3.8 Determination of penconazole (Ofir®) and chlorpyrifos (Dursban®) residues in washing water solution of grape berries



Chapter IV
Discussion and Conclusion

It is well known that systemic pesticides (including chlorpyrifos and 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 both pesticides in the grape leaves and in fruit cortex. The obtained results are in good agreement with those reported by Navarro *et al.*, 2002 on the determination of chlorpyrifos and penconazole in cortical tissues of grape fruit since they reported that chlorpyrifos and penconazole residues were 0.14 ppm and 0.03 ppm, respectively, compared to our results on the residues of both pesticides in the fruit cortex (157.03×10^{-3} ppm for chlorpyrifos and 12.04×10^{-3} ppm for penconazole).

Comparison between the concentrations of chlorpyrifos and penconazole in different parts of the grape (leaves, flesh and cortex) showed higher residues of chlorpyrifos in the three parts of grapevine. This is an evidence that chlorpyrifos molecule is more persistent than penconazole molecule, at least, in grape. These results are in good agreement with the results obtained by Garcia *et al.*, 1999 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 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 organization 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 systemic 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 limit (MRLs) established by different legislations in other countries (e.g. in Spanish and EU legislation MRLs is 0.5 ppm for chlorpyrifos and 0.2 ppm for penconazole in grapes (Oliva *et al.*, 1999). Although the residue levels found in this study are close to those found in other studies, usage of pesticides in our grapevine yards must not be increased. Using other non- pesticide control measures against powdery mildew and berry moth larva 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 pests.

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