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## ESTIMATION OF INSECTICIDE RESIDUE IN DATE PALM FRUITS AFTER CONTROLLING THE RED PALM WEEVIL

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### ABSTRACT

The present work aims to estimate the residue levels in date fruits after three organophosphores (Chlorpyrifos, Diazinon and Fipronil) had been injected into trunk used to control Red Palm Weevil (RPW) of date palm trees. The residue levels of Chlorpyrifos, Diazinon and Fipronil were measured by Gas Chromatography (GC) equipped with Flame Photometric Detector (FPD) and Electron Capture Detector (ECD) after 15 and 30 days from trunk injection, the results showed that their residues were below the maximum residue limits (MRLs). After extraction and clean up procedures, the residue levels of Chlorpyrifos on date fruits was 5.5 ug/kg, which declined to 4.5 ug/kg, following 15 and 30 days of application, respectively (MRL = 50 ug/kg). The initial deposit of Diazinon was 1.2 ug/kg and declined to nondeductible (ND) following 15 and 30 days, respectively (MRL =10 ug/kg). Finally, the Fipronil was ND following 15 and 30 days, respectively (MRL = 5 ug/kg). The above results determined obviously the safest time for harvesting.

**Keywords:** Date palms, Fipronil, Diazinon, Chlorpyrifos, organophosphate, pesticide residues, GC, trunk injection, flame photometric detector, electron capture detector.

### INTRODUCTION

Dates have full nutritive value and are considered to be a principal food in Arab countries, as it is mentioned in the Holy Qura'n. Date palm fruites are very rich in nutritive components, carbohydrates, fats, minerals, proteins, vitamins and dietary fibres, as well as used in folk medicine for treatment of liver diseases and are highly recommended to be consumed by pregnant women before and after delivery. So, with respect to the high consumption of dates in the Middle East, more attention should be given toward health risks due to insecticide residues (Fayadh and Al-Showiman, 1990; Al-Shahib and Marshall, 2003; Al-Hooti et al., 1997).

Date palm insects' infestations can cause dramatic damage to date palm plants. The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is an economically important tissue-boring pest of palms worldwide and one of the most destructive pests of palms in the world (EPPO, 2008, 2009, 2010). The weevil was first described in India as a serious pest of coconut (Lefroy, 1960; Nirula, 1956) and date palms (Buxton, 1918; Raid, 1993).

Several chemical pest control protocols have been recommended for use in Egypt, whereby several pesticides have shown acceptable results to some extent; the most applied pesticides are Chlorpyrifos-methyl, dimethoate, lambda cyhalothrin, thiomethoxam, and primiphos-methyl

etc. Many pesticide application methods have been used, namely, trunk injection, trunk spraying (Hasber, 2012). The pesticide trunk injection technique has many advantages such as minimizing the adverse effects of chemicals, environmental safety, protection of non-target organisms, water savings, and reduction in labor cost (Al Samarrie and Abo Akela, 2011).

People are mainly exposed to pesticide residues through the ingestion of contaminated foods (such as cereals, vegetables, and fruits), which are directly treated with pesticides or are grown in contaminated fields. Therefore, the pesticide residues in food have been strictly regulated by governments of all countries in order to determine whether the concentration of pesticides used exceed their maximum residue limits (MRLs) (European Commission directive, 1993; FAO, 2010). The MRLs are established by each country and sometimes cause conflicts, because different residue levels are acceptable in one country but not in another. Therefore, there is an urgent need to standardize the different MRLs.

The objective of the present work is to determine the residues of three insecticides in date palm fruits after different times from insecticidal application to establish the safest Post-Harvest Interval (PHI) under the weather conditions of examined area, Bahariya Oases, Egypt.

## MATERIAL AND METHODS

### CHEMICALS

Three insecticides were used in field trials at their recommended rates. Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate), Diazinon (O, O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) and Fipronil (5-amino-1-(2, 6-dichloro-trifluoro-p-tolyl) -4-trifluoromethylsulfinylpyrazole -3-carbonitrile) Methanol, have kindly provided by the Egyptian Ministry of Agriculture. Methylene chloride, sodium chloride, anhydrous sodium sulfate, Florisil (60-100 mesh), acetonitrile and hexane were purchased from Sigma – Aldrich, USA.

### FIELD TRIALS

The experiment was carried out at Bahariya Oases, Giza Governorate, Egypt. Date palms, *Phoenix datylefera* var., five palms of 2 to 3 m height were injected once through palm trunk with the manufacturer's recommended concentrations of each insecticide to control the red palm weevil, before 45 days of the harvest. The insecticides were applied into a hole of 15 cm depth, drilled at a horizontal angle of 45° at a height of one meter above the ground. The hole had to be able to hold at least 25 ml of the diluted insecticide. When the injection was over, the hole was closed with a tight-fitting flap. (Mohamed, 2008)

### RESIDUE ANALYSIS

Thirty random samples each of about 50 g of date fruits were collected from palm tree after 15 and 30 days from application (initial deposit time) and were combined in plastic bags. The technique of extraction and clean up procedures were applied as described by (Mollhof, 1975) was adapted and follows. All date fruits were washed with tap water, and the seeds were discarded, 50 g of each sample were randomly selected and mixed with 200 ml distilled methanol in a blender for 3 minutes at high speed and filtered through a dry pad of cotton into a graduated cylinder. A volume of extract (100 ml) was taken and shaken successively with 100, 50, and 25 ml of methylene chloride in a separately funnel after adding 30 ml of saturated sodium chloride solution; then the combined organic phases were dried by filtration through anhydrous sodium sulfate. Thereafter, it was evaporated just to dryness using a rotary evaporator at 40°C. The sample extract was subjected to a column chromatography (250X15 mm internal diameter, i.d.) packed with 6 grams of activated florisil (60-100 mesh) and topped with anhydrous sodium sulfate. The column was eluted with 200 ml eluant (50% methylenchloride - 1.5% acetonitrile - 4.5% hexane) at a rate of 5 ml/min; the collected eluate was concentrated by a rotary evaporator and dissolved in ethyl acetate for residue analysis according to the method of (Mills et al., 1972).

### GC DETERMINATION OF DIAZINON AND CHLORPYRIFOS

The gas chromatograph used was a Hewlett Packard GC Model 6890 equipped with a flame photometric detector (FPD) with a phosphorus filter. A fused silica capillary (Pas-1701) (J&W Scientific, USA) column was used which contained with 14% cyanopropilsiloxane as stationary phase (30 m length X 0.32 mm internal diameter (i.d.) X 0.25 µm thickness).

### GC OPERATION CONDITION

Injector and detector temperatures were 240 °C and 250 °C; initial oven temperature, 180 °C for 2 min, raised by 5°C/min and then held at 260 °C for 2 min, the carrier gas was nitrogen at flow rate of 3 ml/min, hydrogen and air were used for combustion at rates of 75 and 100 ml/min, respectively.

### DETERMINATION OF FIPRONIL RESIDUE

The same gas chromatograph was used but equipped with an Ni<sup>63</sup>-electron capture detector.

### GC OPERATION CONDITION

PAS-S (J&W Scientific, USA) capillary column (30 m length X 0.32 mm internal diameter (i.d.) X 0.25 µm thickness), carrier gas: N<sub>2</sub> at a flow rate of 4 ml/min; injector and detector temperatures were 280 °C and 320 °C, respectively. The initial column temperature was the initial oven temperature, 180 °C for 2 min, raised by 3 °C/min. and then held at 230 °C for 2 min.

### RECOVERY PERCENTAGE

Recovery studies were performed to examine the efficacy of extraction and clean up procedures. Untreated date palm fruits were spiked with a known concentration of pure insecticidal standard solution of each candidate insecticide and extraction and cleanup procedures were performed as above.

## RESULTS AND DISCUSSION

The residues of three insecticides and their recovery % and MRL found in date palm fruits are shown in (Table 1). These residues are found to be under the MRLs level. The optimized experimental conditions were applied to samples to evaluate the efficiency of the method used in the determination of each compound investigated. The average concentrations of Chlorpyrifos were 5.5 and 4.5 µg·Kg<sup>-1</sup> after 15 and 30 days of application, respectively. Diazinon average concentrations were 1.2 and 0.0 µg·Kg<sup>-1</sup> after 15 and 30 days of application respectively. The residue from Fipronil was below the detection limit.

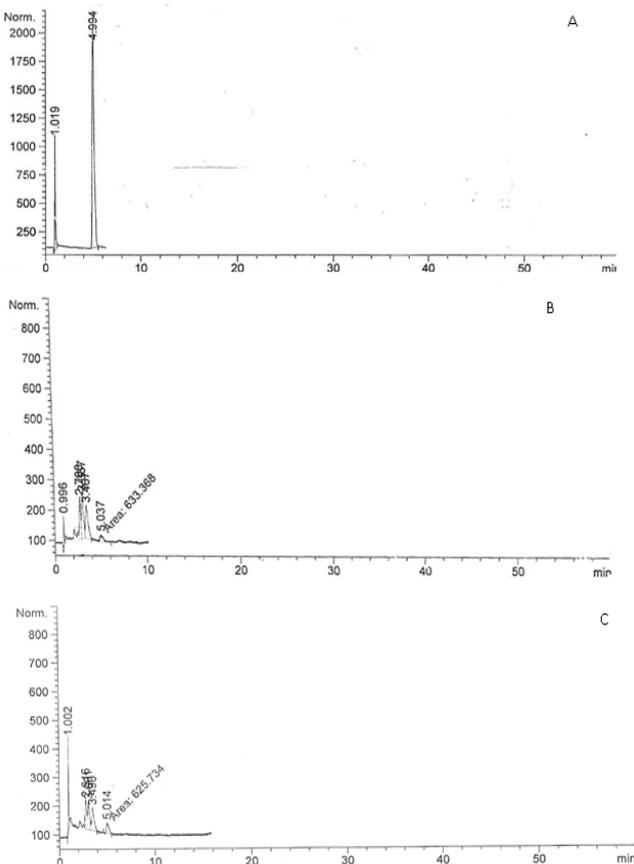
The most frequently used detectors for pesticide residues analysis include Electron Capture Detector (ECD), Nitrogen phosphorus detector (NPD), Flame Photometric Detector (FPD), and MSD. However, it is well known that FPD has been the most used detector in pesticide residues analysis due to its high sensitivity, in particular to halogenated pesticides although all kinds of electron-attracting functional groups such as nitro groups

and aromatic structures also give a response on this detector (Torres et al., 1996; Perret et al., 2002; Stan and Christall, 1991). Figures 1-3, showed GC chromatograms for Chlorpyrifos, Diazinon and Fipronil as standard (A, D, G), residue detected after 15 days (B, E, H) and residue detected after 30 days (C, F, I).

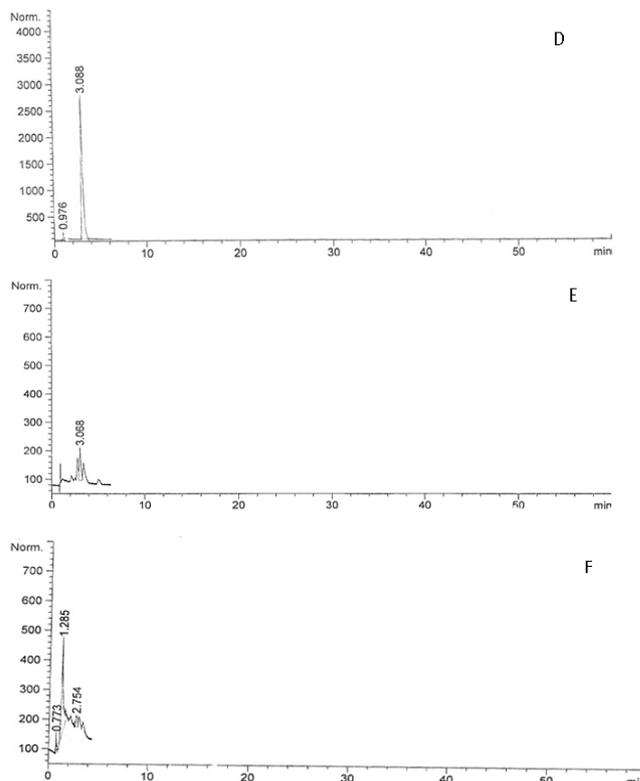
Most countries have established Maximum Residue Limits (MRLs) for pesticides used in the control of pests and diseases not only to safeguard consumer health but also to minimize the presence of these residues in the environment. Data in (Table 2) show MRLs of the selected pesticides in different foods as reported by (Council Directive, 2003; FAO/WHO, 2004). The levels of pesticide residues in foodstuffs are generally legislated so as to minimize the exposure of the consumer to harmful or unnecessary intakes of pesticides, to ensure the proper use of pesticides in terms of granted authorization and registration (application rates and pre-harvested intervals) and to permit the free circulation of pesticide-treated products, as long as they comply with the fixed MRLs.

In Egypt, the MRLs are established by Northwest Horticulture Council through the Program for Analysis of Pesticide Residues in Food (PARA), started in 2001, which monitors the levels of pesticides in fruits, vegetables, and grains consumed. (Gad Alla et al., 2013).

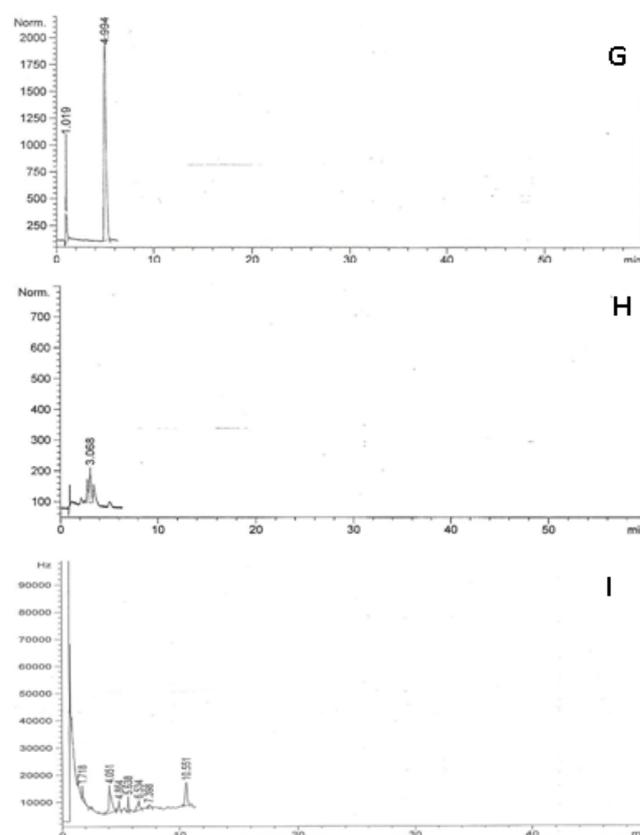
All the data for these insecticide residues were not available during the fruit studies; it was compared with the MRLs established by the European Union (EU) and the USA.



**Fig 1-Shows the chromatograms for standard (A) and samples of Chlorpyrifos after 15 days (B) and 30 days (C) of treatment**



**Fig 2- Shows the chromatograms for standard (D) samples of Diazinon after 15 days (E) and 30 days (F) of treatment**



**Fig 3- Shows the chromatograms for standard (G) and samples of Fipronil after 15 days (H) and 30 days (I) of treatment**

The results in Table 1 show that the residue of Chlorpyrifos after 15 days from application on dates was  $5.5 \mu\text{g}\cdot\text{Kg}^{-1}$  which gradually declined with time reach to  $4.5 \mu\text{g}\cdot\text{Kg}^{-1}$  after 30 days of application. This indicates that after 30 days of treatment, which is only 30 days before the PHI, the amount of Chlorpyrifos was  $4.5 \mu\text{g}\cdot\text{Kg}^{-1}$  and this less than the upper limit of the maximum allowed residue set by the Codex Committee on Pesticide Residues under the Joint FAO/WHO Food Standards Program at  $50 \mu\text{g}\cdot\text{Kg}^{-1}$  for date palm fruits (FAO/ WHO, 1997). After 15 days of treatment, which is 45 days before the PHI, the amount of residual Diazinon was  $1.2 \mu\text{g}\cdot\text{Kg}^{-1}$  which still exceeded the lower limit of the MRL set at  $10 \mu\text{g}\cdot\text{Kg}^{-1}$ , but lay within the upper limit of  $20 \mu\text{g}\cdot\text{Kg}^{-1}$ . At that time the fruit is expected to be ready for sale. Residues of Chlorpyrifos, Diazinon and Fipronil did not exceed the minimum residue limit of 5.5,  $1.2 \mu\text{g}\cdot\text{Kg}^{-1}$  and were non-detectable, respectively.

Few studies on pesticide residues in dates have been carried out. Al-Samarriae and Abo Akela (2011) used fenitrothion, chlorpyrifos and primiphos-methyl to control the lesser date moth by mixing the pesticides with pollen grains at a concentration of 5 mg/kg, and it was found that the residues in dates were at negligible levels at harvest time. With respect to pesticide residues following injection through 3 holes/trunk of 30 ml/plant of a mixture of dimethoate, endosulfan and carbosulfan, the results revealed that dimethoate residue was 0.14 mg/kg at 60 days post injection. This high residue in dates of Khan's results could be related to the high initial injected doses. In a market survey for pesticide residues in dates of Riyadh city markets, the results indicated the persistence of several insecticides, fungicides, herbicides and chlorinated hydrocarbon pesticides and the residues were below and above MRLs, whereas chlorpyrifos was found to be the highest residual detected pesticide ( El-Saeid and Al-Dosari, 2010).

**Table 1: Initial residue deposit and residue decline of Chlorpyrifos, Diazinon and Fipronil in date palm fruits at interval time of treatments**

Insecticides	Amount (ug/kg)		MRL (ug/kg)	Recovery %
	15 days (Mean)	30 days		
Chlorpyrifos	5.5	4.5	= 50 (2010)	93.70
Diazinon	1.2	ND	= 10 (2010)	99.80
Fipronil	ND	ND	= 5 (2010)	88.21

**Table 2: Maximum residual limits\* of three candidate insecticides in different kinds of foods**

Vegetables and Fruits	Chlorpyrifos	Diazinon	Fipronil
Dates	50 ug/kg	10 ug/kg	5 ug/kg
Coconuts	50 ug/kg	20 ug/kg	5 ug/kg
Chestnuts	50 ug/kg	20 ug/kg	5 ug/kg
Vanilla pods	1000 ug/kg	100 ug/kg	5 ug/kg
Pine nuts	50 ug/kg	20 ug/kg	5 ug/kg

\* FAO/WHO, 1993 (Codex Alimentarius Commission)

## CONCLUSIONS

The residue levels of Chlorpyrifos, Diazinon and Fipronil in date palm fruits were measured by Gas Chromatography (GC) equipped with Flame Photometric Detector (FPD) and Electron Capture Detector (ECD), after 15 and 30 days from trunk injection. The results showed that their residues were below the maximum residue limits (MRLs) in both periods, which pointed obviously to the safest time of harvesting.

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