VALIDATION STUDY OF QUECHERS METHOD FOR DETERMINATION OF DIAZINON RESIDUES IN APPLE FRUITS

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Abstract

The present study aimed to throw light on the dissipation and residual levels of diazinon in apple fruits under open field conditions. The pesticide residues found in cultivated fruits from Dakahlia Governorate, Egypt during 2016, using the QuEChERS method prior to analysis by HPLC-DAD. The method validation for extraction and quantitative analysis of diazinon residues in apple fruits using HPLC-DAD, at fortification levels of 0.01, 0.1 and 1.0 mg/kg in apple fruits was used . The obtained results indicated that mean recoveries were ranged from 84.29% to 94.88% with relative standard deviations (RSDs) < 2.30% for HPLC-DAD. In this respect, good linearity was achieved with a determination coefficient (R^2) of 0.999 for the 0.01-5 mg/ L concentration range for HPLC-DAD. The limit of quantification (LOQ) of the method was 0.01 mg/ kg. The results suggest that the diazinon dissipation curves followed the first order kinetics and its half-life was 2.03 days in apple. The residues in apple were below the European Union residue limit (MRL) (0.01 mg/kg) after 10 days of the application.

Key words: Dissipation, Residues, Diazinon, Apple, HPLC-DAD.

1. INTRODUCTION

Pesticides are widely used at various stages of cultivation and during post-harvest storage of fruits and vegetables. Pesticides help to control a wide range of pests and plant diseases that damage crops. Therefore, they play an important role in food production and quality preservation. However, in addition to control pests, pesticides are often harmful to nontarget organisms. Consumers can be injured by the presence of pesticide residues in the agricultural producers, especially when they consumed fresh. The toxic nature of pesticides makes the monitoring of pesticide residues obligatory in food products in order to assess the human exposure to pesticides through foods. Consumers are now much more conscious about the damage caused by pesticide residues. Therefore, there is a growing social desire to reduce the use of pesticides in food and food products and demand for Good Agricultural Practices(GAP). Therefore the concentration of pesticide residues must be monitored not only in various food commodities, i.e. fruits, vegetables, pulses and

cereals, but also in the environment (i.e. soil, air, water, etc.), in order to determine whether they are present at safe levels or not. (Hashem *et al.,* 2013).

The general population is exposed to pesticides on a daily basis via dietary ingestion of contaminated food products. Several studies have indicated that certain foods contain higher levels of pesticide residue, such as fruits, juices, and vegetables (Curl *et al.,* 2003). Vegetables which contain pesticide residue concentrations above the prescript Maximum Residue Limit (MRL) may cause a health hazard to consumers. Fresh fruits and vegetables are important components of a healthy diet, as they are a significant source of vitamins and minerals.

The United Nation's Food and Agriculture Organization (FAO), and the World Health Organization (WHO) have established the Codex Alimentarius Commission (CAC) to monitor and to set up Maximum Residue Limits (MRL's) for pesticides in food. There are limited studies and initiatives taken to assess MRL at local markets in developing countries (Ali and Tahir, 2000).

Pesticide residues analysis in food is one of the most important and challenging tasks in Good Laboratory Practice (GLP). Pesticide residues in crops after application should be studied to obtain more reliable data of the pre-harvest intervals (PHI's). Such periods should also be clarified and recommended to be sure that the residues are below Maximum Residue Limits (MRL's)**.**

The QuEchERS "Quick, Easy, Cheap, Effective, Rugged and Safe" method, which developed between 2000 and 2002 and first reported in 2003, had been widely accepted by the international community of pesticide residue analysts (Payá *et al.,* 2007). The QuEchERS method covers a very wide analyte scope, such as highly polar pesticides, and highly acidic and basic ones. This method involves extraction with acetonitrile and partitioning after the addition of a salt mixture. The final extract in acetonitrile could be directly amenable to analysis.

Apple is a fleshy sweet fruit with many health benefits. It is one of the most important commercial fruit crops which grow on a large scale in Egypt. This crop is attacked by many insects, which require frequent use of pesticides. Organophosphorus pesticides (OPPs) are the most widely used groups of agricultural pesticides. OPPs are one of the most frequently employed worldwide. They are normally sprayed over fruit trees and, as a result of their large production and high stability, they constitute a hazard to the environment and also to human health (OPPs are toxic when absorbed by human organisms because of acetyl-cholinesterase deactivation) (Borges *et al.,* 2009).

Diazinon(*O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) (fig. 1) is non-systemic organophosphorus insecticide and acaricide with agricultural, commercial, and household uses and with contact, stomach, and respiratory action

that can control most sucking and leaf eating insects chewing insects and mites by inactivating neural acetyl cholinesterases. Diazinon is toxic to the nervous system. Diazinon kills insects by interfering with nervous system function, as do all members of the organophosphate chemical family. Normally, impulses are transmitted chemically from the end of one nerve cell to the beginning of another; one of the chemical transmitters used in animal nervous systems is called acetylcholine. After transmitting the nerve impulse, acetylcholine is destroyed by an enzyme called acetylcholinesterase (AChE) in order to clear the way for another transmission. Organophosphates attach to AChE and prevent it from destroying acetylcholine, causing overstimulation of the nerves (Ware, 2000). Diazinon and other organophosphates inhibit numerous enzymes within a wide variety of insects. It was introduced commercially in 1952 and is still commonly used for insect control (Li *et al.,* 2002).

The present study aimed to focus on the dissipation rate and residue levels of diazinon insecticide in apple fruits under field conditions and determine the Pre Harvest Interval (PHI) of apple treated with diazinon.

2. MATERIALS AND METHODS.

2.1. Pesticide used.

Diazinon

(*O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate)
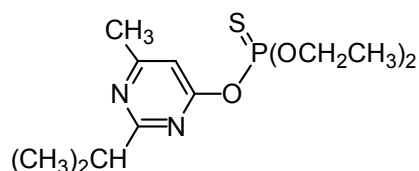

Fig. 1. Structure of diazinon

2.2. Chemicals and Reagents.

All organic solvents used in this study were HPLC grade and supplied by Alliance Bio, USA. Primary and secondary amine (PSA, 40 μm Bondesil) was purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulfate was of analytical grade, purchased from Merck. Sodium chloride was of analytical grade and purchased from El Naser Pharmaceutical Chemicals Company (Egypt). Anhydrous magnesium sulfate and Sodium chloride were activated by heating at 250ºC for 4 hrs in the oven before use and kept in desiccators.

Analytical standards of diazinon (98% purity) was supplied from Central Agricultural Pesticides Laboratory, Giza, Egypt and formulation (Adwizinon 60% EC) was obtained from Wenzhou Lucheng Dongou Dyestuff Intermediate Plant - China.

2.3. Preparation standard solutions of diazinon.

Individual stock solution (100 µg/ml) reference standard of diazinon was prepared in acetoitrile in 100 ml volumetric flask. The successive working dilution and spiking standard solutions for HPLC analysis were prepared daily by diluting the stock solution. All standard and working solutions were stored at 4 ºC.

2.4. Instrumentation.

The chromatographic analyses were preformed using the HPLC system was an Agilent 1260 infinity series (Agilent technologies) equipped with a quaternary pump, variable wavelength diode array detector (DAD), an with an analytical Column: Nucleosil C18 (30 ×4.6 mm (i.d) × 5 um film thickness, auto sample valve. (Chowdhury *et al.,* 2013). The mobile phase was acetonitrile: water (90:10 v/v), while the flow rate was 1 ml/min and the injection volume was 20 µl. the wavelength was 230 nm and the retention time was 2.9 min.

2.5. Field trials:

The field trials, including the dissipation study were carried out at Dakahlia Governorate, Egypt. The plants at fruiting stage were sprayed with (Adwizinon 60% EC) in April 2016 with 300 cm^3 /100 L. water for apple from the commercial products at the recommended dose from the label were used according to pest control program, Ministry of Agriculture and Land Reclamation, Egypt**.** The using knapsack hand sprayer fitted with one nozzle boom. Each treatment consisted of one control plot without application of the pesticide. Replicate samples, two kg apple fruits were collected at random from sampling plots at intervals of one hour after application (zero time), 1, 3,7,10 and 15 days. As soon as the fruits were picked up, and put in polyethylene bags and transferred in ice box to the laboratory. Samples were frozen and then homogenized. The homogeneous matrix was stored in a deep freezer at -20 ºC until the preparation day.

2.6. Analytical method.

2.6.1. Sample extraction and cleanup:

The original QuEChERS method (Anastassiades *et al.,* 2003) was used for sample extraction and clean-up. For the extraction procedure, 10 g (± 0.1) of frozen samples homogenate were weighted into 50 ml centrifuge tubes. And 10 ml acetonitrile were added, the screw cap was closed and the tube vigorously shaken for 1 min using a vortex mixer at maximum speed. After addition of 1 g sodium chloride, 4 g anhydrous magnesium sulfate the mixture was vortexed again for 1 min and then centrifuged for 5 min at 4000 rpm in 5 ºC using a refrigerated centrifuge.

One ml upper acetonitrile layer was transferred to a 15 ml centrifuge tube and clean-up using dispersive solid phase extraction with 25 mg PSA and 150 mg anhydrous magnesium sulfate, the tube was vortexed for 1 min and then centrifuged

for 5 min at 4000 rpm. 1 ml extract was transferred to a vial after filtered through a 0.22 µm PTFE filter (Millipore, Billerica. MA). Then this extract was ready for analysis.

2.6.2. Method validation.

According to SANCO/1257/2013 (SANCO, 2013) within laboratory method validation was performed to provide evidence that the method is fit for the extraction and quantitative determination of diazinon in apple. The method was validated following a conventional validation procedure that included the following parameters: linearity multi-level calibration of diazinon was diluted either with pure solvent in series at (5, 2.5, 1.25, 0.5, 0.1,0.01) µg/ml for HPLC analysis, (matrix effect) comparing the response produced from the diazinon in pure solvent solution with the samples were first extracted and then spiked with diazinon in the same solvent at the same concentration level, (Selectivity and sensitivity) determined limit of quantification (LOQ), trueness (bias) five replicates were used to check the recovery at the levels (1, 0.1, and 0.01) mg/kg and repeatability precision (RSDr).

2.7. Statistical analysis.

The dissipation kinetics of diazinon residues in apple were determined by plotting residue concentration against elapsed time after application, and equations of best curve fit with maximum coefficients of determination (R^2) were determined. For dissipation of diazinon in apple, exponential relationships were found to be applicable corresponding to the general first-order kinetics equation:

$$Ct=C0e^{-kt}$$

where Ct represents the concentration of the pesticide residue at the time of t, $C0$ represents the initial deposits after application and k is the constant rate of pesticide dissipation per day. From this equation, the dissipation half-life periods ($t_{1/2}$=ln 2/k) of the studied insecticide were determined.

3. RESULTS AND DISCUSSION

3.1. Method Validation.

3.1.1. Linearity.

The preparation of calibration curves linearity of diazinon standards were done based on injections of standards diluted with pure organic solvent acetonitrile for HPLC in series at (5, 2.5, 1.25, 0.5, 0.1, 0.01 mg/kg). Standard calibration curve of diazinon was constructed by plotting analyte concentrations against peak areas, Fig. 2. The standard curve equation was:

$y = 167.71x − 2.6967$ with correlation coefficient (R^2 = 0.9999), for HPLC-DAD, where y = peak area and x = concentration (mg/kg).
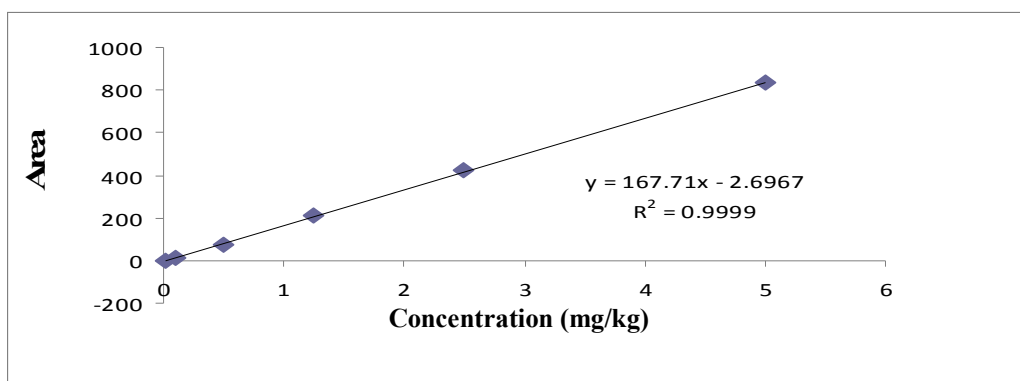
Fig.2. Calibration curve of diazinon with HPLC-DAD analysis.

3.1.2. Limit of quantification LOQ.

The pesticide residue analysis study, the lowest validated level of diazinon with acceptable precision and trueness LOQ was 0.01 mg/kg for HPLC-DAD analysis in apple. According to SANCO/1257/2013 (SANCO, 2013), the LOQ values are acceptable where LOQ ≤ MRL.

3.1.3. Trueness and precision

The trueness or mean of recovery was carried out in 5 replicates at 3 fortification levels (1, 0.1, 0.01 mg/kg) by spiking 10 g of blank samples with standard solution (table1). The obtained mean recoveries range from 84.29% to 94.88% with RSD ranged from 1.22 to 1.94 for HPLC-DAD. According to SANCO/1257/2013 (SANCO, 2013), the obtained mean recoveries were within the acceptable range (70-120%). So, the value confirmed that QuEChERs sample preparation coupled with HPLC-DAD analysis is suitable for the determination of diazinon residue in apple. The repeatability precision (RSDr) involved repeat of recovery levels (1, 0.1, 0.01 mg/kg), five replicates for each level per day on three different days. The (RSDr) value ranged from 1.34 to 2.3 for HPLC-DAD. According to SANCO/1257/2013 (SANCO, 2013) the obtained (RSDr) value was within the acceptable range ≤20% (table1).

Table 1. Mean recovery and repeatability precision of diazinon in apple for HPLC-DAD analysis.

Spiking level (mg/kg) (n*=5)	HPLC-DAD analysis	
	Mean recovery (%±RSD)	RSDr%
0.01	84.29±1.94	2.3
0.1	91.26±1.22	1.34
1	94.88±1.91	2.02

- Number of replicates.

3.2. Determination of diazinon in apple using HPLC-DAD analysis.

The developed method was employed to study the dissipation of diazinon in apple under field conditions with the recommended dose (300 cm³ /100 L).

Dissipation rate of diazinon in apple followed first order kinetics. The initial deposit of diazinon in apple was 2.21 mg/kg, one hour after application. Then gradually decreased to 1.65 mg/kg within one day following application, This value declined to 0.58, 0.23 and 0.01 mg/kg, after 3,7and 10 days after treatment, respectively. Determination coefficient (R^2) was 0.93 which indicated good fitness of regression line to represent residue levels in real field samples. Residue concentration, intercept, slope, R^2, $t_{1/2}$ in apple fruits and estimated PHI values according to European Union regulations are given in (table 2 and Fig 3). Estimated PHI values according to European Union regulations was 10 days for recommended dose. Faster dissipation rate of diazinon in apple may be attributed to environmental conditions in the open field, e.g. higher temperature and exposure to direct sunlight (Fenoll *et al.,* 2009).

Table 2. Residue levels and dissipation behavior of diazinon in apple under field conditions

Intervals after application (days)	Residues (ppm)±SD	% Loss	% Persistence
initial*	2.21 ± 0.18	0.00	100.0
1	1.65 ± 0.06	25.33	74.66
3	0.58 ± 0.03	73.75	26.24
7	0.23 ± 0.02	89.59	10.40
10	0.01±0.006	99.54	0.45
15	0.00	100.00	0.00
RL_{50}	2.03 days		
EU MRL	0.01 mg/kg		
PHI (days)	10 days		

- Initial: one hour after application, RL_{50}: Residual half life, MRL: Maximum Residue limits, PHI: Pre harvest intervals
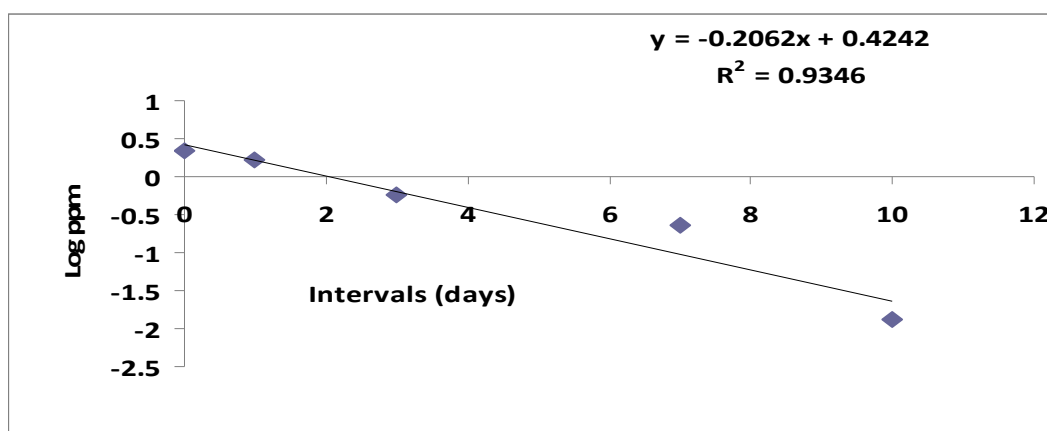

$$y = -0.2062x + 0.4242$$
$$R^2 = 0.9346$$

Fig.3. Degradation lines of diazinon after application in apple under field conditions.

Our results were agreed with (Fardous *et al.,* 2007) who foud that The range of chlorpyrifos, diazinon and carbaryl residues in tomato samples were 0.107- 0.342, 0.157-0.381 and 0.1-0.32 ppm respectively. Also (Fenoll *et al.,* 2006) concluded that multiclass pesticide residues were found in pepper and tomato samples in Spain. Concentrations of 0.1 mg/kg, 0.2 mg/kg and 0.34mg/kg of diazinon, chlorpyrifos and cypermethrin, respectively, were identified in tomato samples. Similarly, multiclass pesticide residues were analyzed and identified in tomato samples in India, finding that some of the samples exceeded the MRL (Karnath 2002 and Kumar *et al.,* 2011).

Degradation and dissipation residues of Diazinon from apple fruits happened because the initial deposits and residues at different intervals of this pesticide are influenced by different factors i.e. evaporation of the surface residue which is dependent on temperature condition, biological dilution which is dependent on the increase mass of fruits, chemical or biochemical decomposition, metabolism and photolysis. Christensen, (2004) reported that the decline of pesticides may due to biological, chemical or physical processes, or if still in the field, due to dilution by the growth of the crop. Plant growth, particularly for fruits is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects (Walgenbach *et al.,* 1991). In addition, the rapid dissipation of originally applied pesticide is dependent on a variety of environmental factors such as sunlight and temperature. However, high temperature is reported to the major factor in reducing the pesticides from the plant surface. Also the light plays an important role in the behavior of pesticide in the environment.

The results of this study are expected to help establish the safe and proper use of diazinon in apple fruits grown in Egypt. Open field studies should be carried out for all pesticiades applied, as different compounds can have dissimilar behavior and affect safety and quality of fruit in a different way.

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دراسة فاعلية طريقة QuEChERS في تقدير
متبقيات مبيد الديازينون في ثمار التفاح

رانيا محمد عبد الحميد – داليا السيد الحفني

قسم متبقيات المبيدات وتلوث البيئة – المعمل المركزي للمبيدات– مركز البحوث الزراعية – الدقي – الجيزة – مصر

تهدف الدراسة إلى إلقاء الضوء علي معدل اختفاء مستويات متبقيات مبيد الديازينون في ثمار التفاح تحت ظروف الحقل المفتوح . وقد أجريت الدراسة علي ثمار التفاح المنزرع في محافظة الدقهلية خلال عام ٢٠١٦. وذلك باستخدام طريقة QuEChERS والتقدير بواسطة جهاز HPLC-DAD. وقد استخدمت طريقة التحقق من الاستخلاص والتحليل الكمى لمتبقيات مبيد الديازينون عند مستويات تلوث ٠،٠٠١ و ٠،١ و ١ مجم/كجم على ثمار التفاح. حيث اظهرت النتائج ان معدلات الاسترجاع تراوحت ما بين ٨٤،٢٩–٩٤،٨٨% بمعامل الانحراف النسبى(RSDs) أقل من ٢،٣٠%. في هذا الصدد لوحظ وجود خطية جيدة بمعامل ارتباط (R²) ٠،٩٩٩، بتركيز يتراوح ما بين ٠،٠١ إلى ٥ مجم/لتر. وكان الحد الكمى للطريقة LOQ(٠،٠١ مجم/كجم). تشير النتائج ان منحنى اختفاء مبيد الديازينون يتبع معادلة الخط المستقيم من الدرجة الأولى وكانت فترة نصف العمر ٢،٠٣ يوما. وكانت متبقيات المبيد في التفاح أقل من الحد الأقصى المسموح به (٠،٠١ ملجم / كجم) بعد ١٠ أيام من المعاملة بالمبيد.

الكلمات المفتاحية: اختفاء – متبقيات – الديازينون –التفاح– كروماتوجرافيا السائل.