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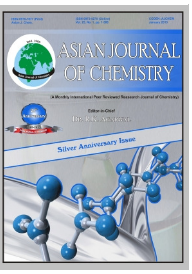

Determination of Diazinon in Fruits from Northeast of Iran Using the QuEChERS Sample Preparation Method and GC/MS

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The aim of this study was to identify pesticide residues found in cultivated fruits from northeastern Iran during 2011, using the QuEChERS method prior to analysis by GC/MS. Selected fruits were apples, grapes, melons, watermelons and cherries that had been sprayed twice with the organophosphorus pesticide, diazinon, during growth. In 75 samples the mean concentration of diazinon ranged from 0.11 to 0.88 mg/kg. The mean level of diazinon in apple samples was below the maximum residue limit recommended by world health and food and agricultural organizations, while the mean levels of diazinon in grapes, melons, watermelons and cherries exceeded the maximum residue limits. These results provide information on the status in one agricultural area in Iran and point to the necessity of controlling the use of some excessively applied and potentially persistent organophosphorus pesticides.

Key Words: Determination, GC/MS, Diazinon, Fruits, QuEChERS.

INTRODUCTION

Pesticides have been used widely in agriculture to combat insects, weeds and moulds for many years, thereby increasing crop productivity. Aside from their positive effects on crop yields, they may also pose various health risks to consumers. Therefore the concentration of pesticide residues must be monitored not only in various food commodities, including fruits, vegetables, pulses and cereals, but also in the environment (in soil, air, water, *etc*.), in order to determine whether they are present at safe levels.

Pesticides comprise a numerous and diverse group of chemical compounds that are used to eliminate pests in agriculture and in households. They improve the yields and quality of crops and food and help to limit the many human diseases transmitted by insect or rodent vectors. In order to get better yields and quality of produce and therefore better incomes, farmers apply pesticides repeatedly during crop growth stages. Organophosphorus pesticides (OPPs) are the most widely used group of agricultural pesticides[1-3]. However, many recent investigations have shown that organophosphorus pesticides can be immunotoxic[4-6], cytotoxic[7-9] and/or genotoxic[10-12]. The potential hazards to health from allowing residues of these pesticides to enter the human food chain underlines the necessity for determination their level in agricultural products and foods. Diazinon (O,O-diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) is an organophosphorus insecticide that can control most sucking and leaf eating insects by inactivating neural acetylcholinesterases in a wide variety of insects. It was introduced commercially in 1952 and is still commonly used for insect control[13].

Several investigations on determination of pesticide residues in wide range of fruits and vegetables[14-19] have shown that the amounts of these residues are often greater than maximum residue level values recommended by European Union, the world health organization and food and agricultural organization. The levels of pesticides in various fruits and crops depend not only on the amount sprayed on the plants but also on the amount present in the soil or in the water used for irrigation. Therefore, some studies have also investigated the levels of various pesticide residues in soil[20,21] and water[22-24] directly. Since biological samples have complex chemical compositions requiring extraction techniques that allow for greater selectivity and concentration of the analytes, preparation of the sample is the most critical step in the determination of low levels of pesticide residues in foods. Conventional methods for the determination of pesticide residues in foods are laborious and time-consuming, requiring considerable amounts of organic solvents and often extracting undesirable interfering substances from the matrix[25].

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method is a sample preparation method used for the extraction of many pesticides in fruits and vegetables with acetonitrile, ethyl acetate or acetone[26-28]. When compared with other sample preparation methods, it is clear that it is extremely fast and inexpensive. It has already received worldwide acceptance because of its simplicity and high throughput enabling a laboratory to process a large number of samples in a short period of time. The method has also proven to be appropriate for GC-NPD or ECD detection[29], which are popular instrumental configurations to monitor pesticides.

In brief the procedure entails the following steps[30-34]:

(i) Weigh 10 g of homogenized sample.

(ii) Add 10 mL acetonitrile and internal standard.

(iii) Agitate intensively.

(iv) Add NaCl, $MgSO_4$ and buffering salts for phase-separation and pH-adjustment.

(v) Agitate intensively and centrifuge.

(vi) Take an aliquot of the upper organic phase and subject it to dispersive solid phase extraction cleanup (d-SPE) by mixing it with $MgSO_4$ and a sorbent (*e.g.* primary secondary amine) to remove water and undesired co-extractives such as sugars and fatty acids.

(vii) Agitate shortly and centrifuge (optionally add analyte protecting agents).

(viii) Analyze the final extract; can be done directly by GC- and /or LC-techniques[27,30,31].

EXPERIMENTAL

Chemicals and samples: Diazinon (O,O-diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) standard was obtained from Dr. Ehrenstorfer GmbH (Ausberg, Germany), with a purity of greater than 93 %. Individual stock solutions of different concentrations were prepared by dissolving diazinon in hexane and storing the solutions in the dark at 4 ºC. Other chemicals were analytical grade reagents. Distilled water was deionized by using a Milli-Q system from Millipore (Bedford, MA, USA). Acetonitrile, magnesium sulphate monohydrate, sodium chloride, sodium hydrogen citrate sesquihydrate, sodium citrate tribasic dehydrate and anhydrous sodium sulphate were from Merck (Darmstadt, Germany). Bondesil-primary/secondary amine (PSA, 40 µm) and graphitized carbon black (Supelclean ENVI-Carb 120/400) were from Varian (Harbor City, CA, USA). A total of 75 samples of apples, grapes, melons, watermelons and cherries were obained from local markets and at various agriculture and gardening sites in Sabzevar, Khorasan Razavi Province, Iran, in 2011. Fruit Samples were stored in the darkness at -18 ºC; they were chopped and homogenized in a laboratory homogenizer at high speed before use.

GC-MS analysis was performed using a Shimadzu QP2010 Ultra series instrument equipped with a split/splitless injector and an Rxi-1 ms capillary column (length 30 m, i.d. 0.25 mm with 0.25-µm film thickness). The carrier gas was helium (1.0 mL/min). The oven temperature program was: from 170 ºC to 230 ºC (2 min) at 5 ºC /min. Injector and detector temperatures were set to 250 ºC and 300 ºC, respectively. The injector was operated in splitless mode. The mass spectrometer was operated in the electron impact (EI) mode with positive ion detection and a source temperature of 200 ºC. The electron energy was 70 eV. Chromatograms were acquired in the scan mode from *m/z* 40 to *m/z* 700 (scan time: 0.9 s, inter-scan time: 0.1 s) or in multiple ion monitoring mode using a dwell time of 0.2 s and a mass span of 0.4 amu. In order to improve Diazinon peak identification, three fragment ions (*m/z* 137, *m/z* 179, *m/z* 304) were monitored. Quantification was performed by extracting a single ion trace (*m/z* 304; the parent ion) from the total ion current chromatogram obtained by multiple ion monitoring.

Analytical procedures: The objective of this study was to determine the level of diazinon in fruit samples obtained by the pesticide residues laboratory. Diazinon was dissolved in hexane at 1000 mg/kg concentrations as stock solutions. Working solutions containing 0.01- 0.5 mg/kg of Diazinon in hexane were prepared from the stock solutions. In order to avoid any influence on the results from possible degradation of pesticides, the working solutions were prepared freshly every day.

Method validation: Correlation coefficients were > 0.99 from 0.030 mg/kg to 0.360 mg/kg for diazinon, indicating good linearity of the method. The limit of quantification of the proposed method was defined as a value 10 times that of background noise. For Diazinon the limit of quantification was below the maximum residue level of each fruit, except for maximum residue levels recommended by European Union. The limit of quantification was 0.05 ppm; the diazinon maximum residue levels for apple, grape, melon (Persian), watermelon and cherry (sweet) for different databases and countries are shown in Table-1[35-37].

TABLE-1
MAXIMUM RESIDUE LIMITS FOR SELECTED FRUITS

Market (database)	Commodities				
	Apple	Grape	Melon (Persian)	Watermelon	Cherry (sweet)
Cod	0.30	-	-	-	1.00
EU	0.01	-	0.01	0.01	0.01
US	0.50	-	0.75	0.75	0.20
Can	0.75	-	0.25	0.25	0.75
Jap	0.10	0.10	0.10	0.10	0.10

Cod: codex alimentarius (international food standards – by the FAO and WHO); US: United States of America; EU: European Union; Can: Canada; Jap: Japan

RESULTS AND DISCUSSION

Diazinon is used worldwide in agriculture for the control of various insects. This compound has a higher acute toxicity than organochlorine pesticides. A study of possible Diazinon contamination of fruits in northeastern Iran was carried out during the crop year 2011. The target fruits were selected from those commonly grown in northeastern Iran. The mean residues levels and standard deviations from the mean for triplicate measurements of fruit samples are presented in Table-2.

Conclusion

The results demonstrate that measurable amounts of diazinon are present in fruits from several locations in northeastern Iran.

TABLE-2
MEAN RESIDUE LEVELS OF SELECTED FRUITS FROM NORTHEASTERN IRAN. THE UNITS GIVEN ARE ppm; STANDARD DEVIATIONS ARE SHOWN IN PARENTHESIS

Fruit	a	b	c	d	e
Apple	0.17 (± 0.04)	0.25 (± 0.08)	0.14 (± 0.04)	0.26 (± 0.11)	0.24 (±0.07)
Grape	0.41 (± 0.15)	0.22 (± 0.08)	0.35 (± 0.12)	0.30 (± 0.14)	0.34 (±0.15)
Melon (Persian)	0.27 (± 0.04)	0.27 (± 0.06)	0.84 (± 0.19)	0.55 (± 0.14)	0.26 (±0.09)
Watermelon	0.12 (± 0.03)	0.20 (± 0.06)	0.18 (± 0.06)	0.23 (± 0.08)	0.17 (±0.07)
Cherry (sweet)	0.18 (± 0.04)	0.11 (± 0.06)	0.30 (± 0.18)	0.88 (± 0.21)	0.12 (±0.02)

a, Afchang village; b, Tabas village; c, Barghamad village; d, Sabzevar local market; e, Neyshabur local market

Routine monitoring of these pollutants in market foods is required for the prevention, control and reduction of pollution, as well as for legal decisions to minimize health risks.

As can seen from Table-2, the mean levels of diazinon in apple samples were below the maximum residue level of 0.30 ppm, recommended by the Codex alimentarius (international food standards- by world health and food and agricultural organizations), US and Canada while it was above the permitted levels in Japan and the European Union. The mean levels of diazinon in grapes were greater than the recommended level of diazinon in Japan. There were no other reports that describe the maximum residue levels for grapes. The Codex does not report any maximum residue level limits for Persian melon, but all mean residue levels were greater than those recommended by the European Union, Canada and Japan. In addition, the Codex did not report a maximum residue level for watermelons, the maximum residue levels for this fruit were greater than the levels permitted by the European Union and Japan, but lower than those for the US and Canada. Finally, the maximum residue levels for sweet cherries sometimes exceeded and sometimes were lower than the values permitted for some countries.

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