Study of Pesticides Residues in Strawberry Fruits Collected from Major Producing Governorates in Egypt

Salim, Y. M. M., E. E. Nour El-Deen, and A. M. K. Nassar*
Pesticides Chemistry and Toxicology, Plant Protection Department, Faculty of Agriculture, Damanhour University, Damanhour, El-Beheira PO Box 59, Egypt

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Abstract: Strawberry is an important crop in Egypt. It's been bombarded with tones of pesticides to control various insects and pathogens. In the present study, pesticides residues were evaluated in strawberry samples that collected from different places, El-Beheira (Al-Nubaryia, Al-Mahmoudia, and Bader cities), El-Minofyia, and Ismailyia Governorates. Pesticides residues were extracted and cleaned up with QuEChERS (quick, easy, cheap, effective, rugged, and safe) method. Then extracts were analyzed with GC-MS to determine the pesticide residue levels. Results showed that the average recovery percentages ± relative standard deviation (RSD) of pesticides from fortified strawberry samples ranged from 91.7±4.6 to 102.1±5.2% and 92.8±6.2 to 102.3±4.0% at 0.1 and 1 μgL^{-1}, respectively. Residue levels of boscalid, p,p-DDD, and propiconazole pesticides in strawberry samples from Bader city were 5.000, 0.104, and 0.048 $mgKg^{-1}$, respectively. Endrin and propiconazole residue levels were detected in samples from El-Minofyia city at 0.098 and 0.038 $mgKg^{-1}$, respectively. On the other hand, Propiconazole residues were found in all strawberry samples from the five locations under investigation. Residues of detected pesticides were less than their corresponding maximum residue limits of European Union (EU-MRLs) that highlight the safety of marketed strawberry for Egyptian consumers.

Keywords: Pesticide Residues, Strawberry, QuEChERS, GC-MS, Endrin, Propiconazole, Boscalid

INTRODUCTION

Strawberry is an important cash horticultural crop in Egypt with about 5 and 75% of the World and African production, respectively (FAO 2018). It is an important cash fruit for Egyptian exporters. Most importantly, it is a rich source of vitamins (A, B_1, B_2,B_6, C, and E), minerals (Fe, Ca, P, Zn, Cu, K, Mn, Na, and Se), carbohydrates, fiber, protein, organic acids, and polyphenols (anthocyanins, anthocyanidins, carotenoids, flavonoids, furan fatty acids, and hydroxybenzoic and hydroxycinnamic acids) (Belitz et al. 2009). Therefore, consumption of strawberry provides human with numerous health benefits against cardiovascular, neurodegenerative, aging, obesity, and cancer (Saber *et al.*, 2016). Although it's highly popular fruit among consumers, it's been bombarded with tonnes of pesticides to control various pathogens including insects, mites, fungi and bacteria (Zalom *et al.,* 2006).

Accordingly, monitoring pesticides residues in strawberry has been conducted routinely on national and international basis. Most of results confirmed with the presence of elevated levels of pesticides in strawberry compared to the permitted amounts (Belitz *et al.*, 2009). For example, chlorfluazuron, penconazole, and pyrimethanil insecticides were in greater amounts, while α- and β-endosulfan, chlorpyrifos, carbofuran, triadimenol I and II, captafol-metabolite, and iprodione were less than maximum residue limits (MRLs) in strawberry samples that were collected from Gaza Governorates and measured using GC-MS (Safi *et al.*, 2002). Myclobutanil, dichlorvos, and fenarimol were detected out of 350 pesticides searched for in strawberry among other produce samples (Ueno *et al.*, 2003). In a multi-country surveillance study, 593 samples of strawberry were analyzed for pesticides residues: 93% of them confirmed with pesticides residues with cyprodinil, fludioxonil, fenhexamid, tolylfluanid, and azoxystrobin being the most detected (Looser *et al.*, 2006). In a 10 years residue monitoring study in Brazil, carbendazim and chlorpyrifos were the detected pesticides in 992 samples of strawberry with levels exceeding the MRLs (Jardim and Caldas, 2012). Chlorpyrifos, chlorpyrifos-methyl, L-cyhalothrin, and profenofos concentration in strawberry were equal to or greater than EU-MRLs (Ahmed *et al.*, 2014). Similarly, pyrimethanil, cyprodinil, fludioxonil, and boscalid were the most detected pesticides in great amounts compared to the tolerable limits (Sójka *et al.*, 2015). However, residues of pesticides in strawberry were reported to be negligible for pyrimethanil (Malhat *et al.*, 2014) and hexythiazox (Saber *et al.*, 2016).

Pesticides residues analysis depends mainly on the employed methods for extraction and determination. Primarily, the extraction technique of pesticide residues is critical (Lehotay, 2011). Therefore, the majority of researchers and certified agencies recommend the usage of QuEChERS (quick, easy, cheap, effective, rugged, and safe)method for extraction and cleanup (Anastassiades *et al.,* 2003c; Frenich *et al.*, 2008; Lehotay, 2011; Schreiber *et al.*, 2013; Sójka *et al.,* 2015). The QuEChERS extracts were analyzed by LC-MS, LC-MS/MS, GC-MS (EI, CI, TOF), GC-ECD and GC-NPD (Looser *et al.*, 2006). Gas chromatography with nitrogen–phosphorus and flame photometric detection was used to estimate nitrogen- and phosphorus-containing pesticide residues in vegetables (Anastassiades *et al.*, 2003c; Ueno *et al.*, 2003). Gas chromatography coupled with mass spectrometry (GC-MS, GC/MS/MS) technique is widely used technique to estimate multi residues of pesticides (52 compounds) in strawberry (Bolanos *et al.*, 2007). Also, UPLC-MS/MS after QuEChERS extraction was effective in the determination of 53 pesticide residues in various samples including

*Corresponding author e-mail: atef.nassar@dmu.edu.eg

strawberry (Frenich *et al.*, 2008). A validated GC/MS/MS method was applied after QuEChERS extraction of 151 pesticide residues in strawberry. Also, about 27, 143, and 600 pesticides were measured directly using LC-MS/MS, low pressure GC-tandem mass spectrometry (LP-GC-MS/MS), and targeted and untargeted analysis using two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF-MS), respectively (Fernandes *et al.*, 2014). The UHPLC and GC-MS were employed in the survey of 121 samples for pesticide residues in Poland (Sójka *et al.*, 2015).

Consequently, contamination of strawberry fruits with pesticides residues raises serious health concerns for consumers. Therefore, observing residue amounts must beroutinely conducted by national institution before exporting or importing strawberry. Thus, the current research project aimed to detect pesticides residues in strawberry fruits that were collected from major production areas in Egypt specifically from El-Beheira (El-Nubaryia, Al-Mahmoudia, and Bader Cities), El-Minofyia, and Ismailyia Governorates.

MATERIALS AND METHODS

Chemicals and pesticide standards

Pesticides reference standards of >99% purity (Aldrin (10; LOQµg Kg^{-1}), Atrazine (10), Azinophos-methyl (20), α-BHC (15), Δ-BHC (10), γ-BHC (25), Boscalid (25), Bromuconazole (30), Butralin (20), Cadusafos (50), Chlorfluazuron (50), Chlorpyrifos (10), Chlorothalonil (25), Cyfluthrin (15), λ-Cyhalothrin (10), Cypermethrin (25), pp-DDD (20), pp-DDE (10), pp-DDT (10), Deltamethrin (15), Diazinon (10), Difenoconazole (20), Dimethoate (50), Dieldrin (5), Endosulfan (15), Endrin (50), Ethoprophos (5), Esfenvalerate (10), Ethion (5), Fenamiphos (15), Fenarimol (10), Fenpropathrin (25), Heptachlor (10), Heptachlor-epoxide (10), Malathion (10), Methoxychlor (25), Myclobutanil (15), Propiconazole (15), Pendimethalin (50), Permethrin (10), Phenthoate (15), Pirimiphos-methyl (10), Profenofos (15), Prothiophos (10), Pyridaben (10), Quinalophos (15), Tebuconazole (15), Thiamethoxam (50), Thiocyclam (25), and Triazophos (10)) were purchased from Cornell Lab, Cairo, Egypt. Stock solutions of100 µgmL^{-1} were prepared and working standard pesticide mixtures of50 and 10 µgmL^{-1} were prepared in acetonitrile (MeCN). Triphenyl phosphate (TPP), MeCN, acetone, glacial acetic acid, and QuEChERS extraction and dispersive SPE clean-up kits (Agilent Technologies catalogue # 5982-0650 and 5982-5056, respectively) were purchased from Arabian Group for Integrated Technologies (AGITECH), Cairo, Egypt. Limits of quantification (LOQ) of pesticides were evaluated following the directions of US EPA (1995), EC (2004), ICH (2005), EC (2007), OCED (2007), and USP (2010) and based on a standard curve of each pesticides.

Strawberry samples

Fresh strawberry samples were collected from farms at El-Beheira (El-Nubaryia, Al-Mahmoudia, and Bader cities), El-Minofyia, and Ismailyia Governorates from patches ready for distribution to local market. Five samples from each farm consisting of 2 kg each were transferred to the laboratory, thoroughly mixed, and chopped into small pieces using an electrical blender at high speed. Then three sub-samples of 10 g from each sample were extracted, cleaned-up, and analyzed for pesticide residues.

Extraction of pesticide residues using QuEChERS method

Extraction and cleanup were accomplished following a modified method of Anastassiades *et al.* (2003a) using extraction and dispersive SPE clean-up kits (Agilent Technologies catalogue # 5982-0650 and 5982-5056, respectively). About 10 g of homogenized strawberry samples were transferred into 50 mL Falcon tube. Then 10 mL of 0.1% acidified MeCN were added. Tubes were agitated vigorously for one min by using Vortex mixer at maximum speed and four g of anhydrous $MgSO_4$ and one g NaCl were added to each tube and mixed on Vortex mixer immediately for one min. A 50 µL of TPP (internal standard) solution was mixed for 30 seconds, and extracts were centrifuged for 10 min at 4000 rpm (Hermle Labortechnik GmbH, Siemensstr.25 D-78564 Wehingen, Germany).

Approximately, one mL of the upper MeCN layer was aliquoted into 15 mL Falcon tubes containing 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$, capped tightly and shaken by hand for 5 min. Samples were centrifuged for 5 min at 4000 rpm. Samples were extracted and cleaned up in triplicates. About 0.5 mL of extracts was transferred into amber HPLC vials for GC/MS analysis.

GC/MS running conditions

Samples were analyzed with Agilent Technologies gas chromatography system model 7890B coupled with mass spectrometry model 5977A (GC/MS) instrument and an autosampler (Agilent, Little Falls, DE). The system was equipped with a split/splitless injection inlet, and electronic pressure control (EPC). The system was controlled by MSD Chem Station software (version F.01.03.2357) and data analysis was done by Mass Hunter GC/MS acquisition software (version B.07.03.2139). Extracts and recovery samples (2 µl) were injected in the GC/MS system in splitless mode. An HP-5MS capillary column (30 m X 0.53 mm i.d. 0.25 um film thickness) was used to separate the components. Helium was used as the carrier gas. Separation conditions were according to AOAC (2007) as the following program: initial column temperature set at 80°C for 6 min. It was increased to 215°C at 15°C/min (hold for 1 min), then to 230°C at 5°C/min and finally to 290°C at 5°C/min (hold for 2 min). The carrier gas was at a constant flow rate of 1.1 ml/min. The target compounds were identified by their full mass spectra scans and retention time using the total ion current as a monitor to give a Total Ion Chromatogram (TIC). The use of the full scan mode allowed the contrast of the spectrum of obtained compounds with the EI-MS library.

Quality assurance

The quality assurance criteria were accomplished following the criteria of Codex Alimentarius committee. Calibration curves for HPLC and GC/MS systems were made at levels of 10, 20, 50, and 100 µg L^{-1}. Calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of internal standard (IS)) to the relative concentration of analytes (concentration of analyte/concentration of IS). The regression equations of standards were used to calculate amounts of detected pesticides. Recovery and reproducibility were evaluated by spiking pesticide standards to fruit samples at levels of 1, 10, and 50 µg L^{-1} and each concentration was repeated three times. The average recovery varied between 81–102% and 88–98% for the GC/MS and HPLC, respectively.

Method and machine precision

Precision of the analytical method was confirmed through the repeatability (intra-day assay) and intermediate precision (inter-day assay) (Ermer 2005). The intra-day and inter-day precision of the method were determined by repeating the analysis of recovery samples on same day and over five consecutive days, respectively. The precision was expressed as coefficient of variation percentages ((mean/standard deviation) × 100). The limits of quantification (LOQs) were calculated from the signal-to-noise (S/N) ratios of the samples with the lowest concentration level of some studied pesticides.

RESULTS AND DISCUSSION

Data in Table (1) showed inter–assay and intra-assay precision, which were obtained from the analysis of fortified samples with standards of pesticides. Results indicated that the intra- and inter-assay values ranged from 4.45 to 5.63 and 4.93 to 8.12%, respectively. Also, the recovery percentages ± relative standard deviation (RSD) were ranged from 91.7±4.6 to 102.1±5.2% and 92.8±6.2 to 102.3±4.0% at 0.1 and 1 µgL^{-1} levels, respectively. The results of accuracy, recovery, and RSD met acceptable criteria according to World Health Organization (2014) requirements.

Moreover, RSD values were below 20%, which indicate the suitability of employed method in the analysis of pesticides residues in investigated strawberry samples. Accordingly, all data of residue analysis were corrected according to these obtained recovery percentage values.

The developed analytical method was applied for analysis of pesticides residue in strawberry fruits collected from farms at El-Beheira (El-Nubaryia, Al-Mahmoudia, and Bader cities), El-Minofyia, and Ismailyia Governorates. QuEChERS technique was applied for the determination of pesticide residues in strawberry fruits because of its great simplicity and sensitivity (Lorenz *et al.*, 2014). This method covers a wide range of pesticides (polar, pH-dependent compounds), simple (no laborious steps, minimal sources of errors) and cheap (Wilkowska and Biziuk, 2011). The solvent consumption is low (10 mL acetonitrile, GC- and LC-amenable) and practically no glassware is needed (Anastassiades *et al.*, 2003b).

Table (1): Coefficients of variation (CV %) and recovery percentages ± relative standard deviation (RSD) values of some pesticides that were extracted from spiked strawberry samples and analyzed using GC/MS

Pesticide	CV* (%)		Recovery (%) ± RSD	
	Intra-Assay	Inter-Assay	0.1 µgL^{-1}	1 µgL^{-1}
Heptachlor	5.18	7.62	91.7±4.6	92.8±6.2
Dieldrin	4.45	6.54	96.7±2.0	99.1±1.2
p,p-DDD	4.50	7.69	93.5±2.1	95.6±1.5
p,p-DDT	5.36	6.34	98.2±3.5	102.3±4.0
Methoxychlor	5.53	4.93	97.1±2.9	99.3±4.0
Chlorpyrifos-methyl	4.83	6.84	97.5±3.1	99.7±3.7
Malathion	4.96	8.12	102.1±5.2	101.1±2.9
Chlorothalonil	5.55	7.91	98.2±4.1	99.0±3.4
Propioconazole	4.93	6.25	94.5±3.6	97.0±5.7
L-Cyhalothrin	5.63	8.01	93.7±4.5	96.1±5.1

*Inter– and intra-assay precision data obtained from the analysis of the concentrations of each standard pesticide in extracts of fortified strawberry samples

Results presented in Table (2) showed the residue levels of the detected pesticides in strawberry samples, which collected from different places in Egypt. These results indicated that boscalid, p,p-DDD, and propiconazole pesticides were detected in strawberry samples from Bader city at 5, 0.104, and

0.048 $mgKg^{-1}$, respectively. Endrin and propiconazole pesticides were detected in samples from Al-Minofyia city at 0.098 and 0.038 $mgKg^{-1}$, respectively. Whereas, Propiconazole was found in strawberry samples collected from all five locations under investigation at levels less than the MRL according to European Union (EU); 0.01, 0.05, 0.01, and 6.0$mgKg^{-1}$ofendrin, p,p-DDD, propiconazole, and boscalid, respectively. These results were in agreement with Chen *et al.* (2007) who determined residue of boscalid in cucumber with GC–ECD and Hiemstra and De-Kok (2007) whom reported a multi-residue method for targeted analysis of pesticides (including boscalid) in crops using liquid chromatography–tandem mass spectrometry. Although the absence of DDT in all samples of strawberry, its metabolite p,p-DDD was reported in great concentration in Bader city. This may be due to the different mechanisms of degradation such as microbial effect and oxidation (Matsumura *et al.,* 1972), photoionization, electron transfer (Bowma and Sans, 1980) (Laymann *et al.,* 1990) and aerobic biodegradation.

Table (2): Limits of Quantitation (LOQ), maximum residue limits (MRLs) according to the European Codex Alimentarius guidelines, and levels of detected pesticides ($mgKg^{-1}$) in strawberry samples collected from different places in Egypt using GC-MS

Pesticide	LOQ	MRLs	BA	NU	MA	ME	IS
Boscalid	0.025	3.000	5.000	-	-	-	-
Endrin	0.050	0.050	-	-	-	0.098	-
pp-DDD	0.020	0.200	0.104	-	-	-	-
Propiconazole	0.015	0.100	0.048	0.060	0.022	0.038	0.020

Bader (BA), El-Nubaryia (NU), Al-Mahmoudia (MA), El-Minofyia (ME), and Ismailyia (IS) cities in Egypt

CONCLUSIONS

Pesticides residue levels of p,p-DDD, Endrin, propiconazole, and boscalid in all strawberry samples were less than the permissible maximum residue limit (MRL) compared to the proposed by Standards of European Union. Therefore, no health risk associated with human consumption of strawberry from studied locations.

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Conflict of Interest

The authors declare no conflict of interest.

Co-Authors Contributions

Y.M.M.S.: proposed the research problem, collected strawberry samples, extracted the samples for GC-MS analysis, and revised the manuscript, **E.E.N.E.**: proposed the research problem, run the quality control samples in GC-MS equipment, and wrote the manuscript, and **A.M.K.N.**: proposed the research problem, conducted GC-MS analyses, run statistical analysis of results, and wrote and submitted the manuscript.

دراسة متبقيات المبيدات في عينات الفراولة المجمعة من أكثر المحافظات المصرية إنتاجا

يحيي محمد محمد سالم، إيمان السيد نور الدين، عاطف محمد خضر نصار
قسم وقاية النبات، كلية الزراعة، جامعة دمنهور، دمنهور، محافظة البحيرة، ص ب ٥٩ مصر

تم جمع عينات الفراولة من أماكن الإنتاج والتسويق بكل من محافظات البحيرة (مدن: النوبارية ، بدر ، المحمودية) والمنوفية والإسماعيلية. وتم إستخدام طريقة الـ QuEChERS (quick, easy, cheap, effective, rugged, and safe) لاستخلاص وتنقية العينات لمتبقيات المبيدات. تراوحت نتائج فحص العينات باستخدام جهاز كروماتوجرافي الغاز وكاشف مطياف الكتلة (GC-MS) لنسب الـ Recovery ± RSD بين ٩١.٧±٤.٦ و ١٠٢.١±٥.٢٪ عند مستوي ٠.١ ميكروجرام/لتر وبين ٩٢.٨ ± ٦.٢ و ١٠٢.٣±٤.٠٪ عند مستوي ١ ميكروجرام/لتر، علي التوالي. أوضحت النتائج تواجد متبقيات مبيدات الـ Boscalid، p,p-DDD ، Propiconazole في عينات الفراولة المجمعة من مدينة بدر بمقدار ٠.٥٠٠ ، ٠.١٠٤ ، و ٠.٠٤٨ مجم/كجم، علي التوالي. أيضا تم رصد متبقيات مبيدي الـ Endrin و الـ Propiconazole في عينات المنوفية وتراوحت بين ٠.٠٩٨ و ٠.٠٣٨ مجم/كجم من، علي التوالي. كما تم رصد متبقيات مبيد الـ Propiconazole في جميع عينات ثمار الفراولة المختبرة وأن كانت النتائج المتحصل عليها أقل من القيم الموثقة بدستور الأغذية للإتحاد الأوروبي EU-MRLs مما يوضح أمان وسلامة الاستهلاك الغذائي للفروالة المتواجدة في الأسواق المصرية.