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Determination of Pesticides Residues in Orange Samples Marketed in Assiut Governorate, Egypt

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ABSTRACT

Orange is one of the most important citrus crops in Egypt. It subsists plentiful of impact issues such as pesticide residues regardless of the unwise use of pesticides. Herein, determination and quantification analyses of pesticide residues were determined by using LC-MS/MS and quick, easy, cheap, effective, rugged, and safe (QuEChERS) method in orange samples marketed in El Badari, Assiut city, Manfalut, El Qusiya, and Dairut that located in Assiut governorate, Egypt. As a result, 14 pesticide residues were found in total. one pesticide residue exceeded the maximum residue levels (MRLs) in two cities, i.e. El Badari and El Qusiya, (Flusilazole 0.02 and 0.03 mg/kg, respectively). Pyridaben was the most frequently found pesticide residue in 3 cities (Assiut city, Manfalut, and Dairut). Further, there was no health risk index appeared among the pesticide residues. Consequently, a regular pesticide residue analysis program is recommended to monitor pesticide residues in orange samples to retain the food safe, especially in upper Egypt.

INTRODUCTION

Pesticides are a substantial tool in pest control (Ahmed *et al*., 2019; Wang *et al*., 2020). However, the unwise use of pesticides leads to numerous issues (Li *et al*., 2020a). Pesticide residue is considered one of the serious problems that confront the agricultural sector (Ahmed *et al*., 2019; Camara *et al*., 2020; Li *et al*., 2020b). Further, it usually poses complications to human health attention. However, the existence of pesticides in foods, especially fruits, with levels over the maximum residue levels (MRLs), has long-term counteractive effects on the ecosystem, public health, and national income (Ahmed *et al*., 2019). Unfortunately, not all growers follow the legal practices with pesticides during production (Biziuk and Stocka 2015; Ahmed *et al*., 2016). In this interim, pesticide residues analysis was considered paramount and must be continued (Ahmed *et al*., 2014a,b). In this study, the contamination levels of pesticide residues in orange samples were marketed in five cities in Assiut governorate (El Badari, Assiut city, Manfalut, El Qusiya, and Dairut) were determined using LC-MS/MS.

MATERIALS AND METHODS

Sample Collection and Preparation:

Five samples (2 kg each) of orange were collected from different cities in Assiut, Egypt (Table 1). Each sample was cut into small pieces and well mixed then wrapped with polyethylene bags and placed into a deep freezer with labels. Samples were left 24 hours before getting them out of the deep freezer and grinding them with a grinder, then 10gm of each sample were weighed three times and kept in 50 ml falcon tubes, afterwards samples were turned into the deep freezer till the extraction.

Table 1. Random markets from which the orange samples obtained

No.	Market	City
1	El Badari	Assiut
2	Assiut city	Assiut
3	Manfalut	Assiut
4	El Qusiya	Assiut
5	Dairut	Assiut

Sample Extraction:

Samples were extracted with acetonitrile, QuEChERS extraction salts, and water if necessary. Ten ml of acetonitrile were added to each sample and samples were then shaken by hands for a minute and vortex for a minute too, afterwards 1 bag of salts was added to each sample and shaken with hands and vortex. Then samples were centrifuged at 5000/5 RPM. One milliliter from the upper layer of the sample was taken and filtered with a 0.22 filter. Acetonitrile purchased from sigma Aldrich, and QuEChERS salts purchased from Agilent Technologies.

Preparation of Pesticide Standards:

Stocks standards were received in the ampoule and stored in an appropriate glass container. Primary stock solutions were prepared from pure reference materials. Working solutions were prepared from the stock solutions or other working solutions. 100 µl of stock Standard solution were each diluted to 1 ml. The working St. solutions were prepared by pipetting an accurate amount of the stock solution into an appropriate volumetric flask and then diluted.

Instrument Condition:

LC-MS/MS System 6500 QTRAP, Applied Biosystem equipped with Ekspert UPLC system. The column: Phenomenex Analytical HPLC column Luna® with 3 µm C18 100 Å, LC, column 50 × 2 mm (Internal diameter) and the column temperature was 40°C. Mobile phase A: 2 mM ammonium formate in water. Mobile phase B: methanol. Injection volume: 2 µl, autosampler temperature: 4°C, source temperature: 400°C. Ion spray potential was 5500 V, and the mode electron spray ionization was the positive mode.

Method Validation:

In this study, results were revealed as mg/kg (Table 2). Replicate measurements of lowest concentrations spiked test samples at least 5 times. Lowest spike level 0.01 mg/kg meeting the method performance criteria for trueness (mean recoveries are within the range 84–99% (Acceptable range 70–120%) and precision (repeatability RSD ≤ 20 %). LOD ranged from 0.000621 to 0.001578 mg/kg and LOQ ranged from 0.00141 to 0.00526 mg/kg. It has been noted that LOQ < MRL.

Table 2. The average recovery percentage (spike level 0.01 mg/kg) and other validated parameters of analytes in orange samples

Analyte	Average determined value for 5 replicates (mg/kg)	Average recovery %	SD	RSD%*	LOD = 3*SD mg/ kg	LOQ = 10*SD mg/ kg
Acetamiprid	0.00824	92%	0.000365	4%	0.001095	0.00365
L-cyhalothrin	0.00790	99%	0.000141	2%	0.000423	0.00141
Flusilazole	0.00790	89%	0.000224	3%	0.000672	0.00224
Dimethoate	0.00718	92%	0.000311	4%	0.000933	0.00311
Imidacloprid	0.00738	84%	0.000526	7%	0.001578	0.00526
Fenpyroximate	0.00804	98%	0.000336	4%	0.001008	0.00336
Hexythiazox	0.00678	87%	0.000377	6%	0.001130	0.00377
Pyridaben	0.00844	95%	0.000251	3%	0.000753	0.00251
Fenamiphos	0.00668	94%	0.000259	4%	0.000777	0.00259
Spirodiclofen	0.00846	94%	0.000207	2%	0.000621	0.00207
Clofentezine	0.00874	95%	0.000270	3%	0.000810	0.00270
Thiamethoxam	0.00872	95%	0.000249	3%	0.000747	0.00249
Triticonazole	0.00724	94%	0.000305	4%	0.000915	0.00305
Propiconazole	0.00886	94%	0.000378	4%	0.001134	0.00378

*RSD% = (SD/Mean) x 100

RESULTS AND DISCUSSION

The data in Table 3 represent the level of pesticide residues in orange samples. Fourteen pesticides (insecticides, nematicides, fungicides, and acaricides) residues were detected in the study locations. The most frequent pesticide residues were pyridaben (Assiut city, Manfalut, and Dairut markets), acetamiprid (El Badari and Manfalut), flusilazole (El Badari and El Qusiya), and fenamiphos (Assiut city and Manfalut). However, flusilazole was considered the only pesticide residue that exceeded the MRL level in two cities (El Badari and El Qusiya) (0.03 and 0.02 mg/kg whereas the MRL was 0.01 mg/kg).

Further, it was significant to calculate the estimated daily intake (EDI) in order to assess the realistic estimate of pesticide residue based on the perspective of international guidelines **(FAO, 2009)**. Importantly, it is expressed as a microgram of pesticide per kilogram body weight per day (µg/ kg b.w./ day) and was calculated from the following equation:

$$\mathbf{EDI = \Sigma\ C\ x\ F / D\ x\ W}$$

Where C is the sum of the concentration of pesticide in each location (µg/kg), F is the mean annual intake of food per person, D is the number of days in a year (365), and W is the mean body weight (80 kg). The annual intake per person of orange in Egypt is 10.4 kg/person/year (Capmas, 2017).

Furthermore, the health risk index (HRI) was deemed the proportion of the estimated daily intake (EDI) to the accepted daily intake (ADI). However, the ADI values were obtained from the European Union Pesticides Database (2009), whereas EDI clarified if the calculated amount of pesticide residues be greater than the amount of the pesticide that can be consumed every day for the lifetime. Interestingly, HRI value that exceeded the value of one is considered to be risky for human health (Ahmed *et al*., 2019).

Data in Table 4 demonstrated the EDI values of the pesticide residues and their health risk index in the orange samples. None of HRI values were found to be greater than one. Therefore, none of the pesticide residues found in selected markets is considered a health risk issue in the study locations in Assiut governorate.

Table 3: Level of pesticide residues in Orange samples from different markets in Assiut Governorate

Market	Pesticide	A.I. detected (mg/kg)	U MRL(mg/kg)
El Badari	Acetamiprid	0.71	0.9
	L-cyhalothrin	0.12	0.2
	Flusilazole	0.02	0.01
	Dimethoate	0.01	0.01
	Imidacloprid	0.8	1
Assiut city	Fenpyroximate	0.04	0.05
	Hexythiazox	0.45	1
	Fenamiphos	0.01	0.02
	Pyridaben	0.22	0.3
Manfalut	Acetamiprid	0.87	0.9
	Fenamiphos	0.01	0.02
	Pyridaben	0.2	0.3
	Spirodiclofen	0.4	0.5
	Clofentezine	0.09	0.5
El Qusiya	Clofentezine	0.1	0.5
	Thiamethoxam	0.06	0.15
	Flusilazole	0.03	0.01
Dairut	Triticonazole	0.01	0.01
	Pyridaben	0.2	0.3
	Propiconazole	3	9

Table 4. Acceptable daily intake (ADI), estimated daily intake (EDI), and Health risk index (HRI) for pesticide residues found in orange samples.

Pesticide	AD (µg/kg, b.wt/day)	EDI (µg/kg, b.wt/day)	HRI (EDI/ADI)	Health risk
Acetamiprid	25	0.562	0.0225	No
Cyhalothrin	2.5	0.042	0.0170	No
Flusilazole	7	0.017	0.0025	No
Dimethoate	2	0.003	0.0017	No
Imidacloprid	60	0.284	0.0047	No
Fenpyroximate	10	0.014	0.0014	No
Hexythiazox	30	0.160	0.0053	No
Fenamiphos	0.8	0.007	0.0089	No
Pyridaben	10	0.220	0.0220	No
Spirodiclofen	15	0.142	0.0094	No
Clofentezine	20	0.067	0.0033	No
Thiamethoxam	26	0.021	0.0008	No
Triticonazole	25	0.003	0.0001	No
Propiconazole	40	1.068	0.0267	No

Generally, numerous studies were carried out to investigate the pesticide residues in orange samples. Badr *et al*., 2019 found that more than 50 pesticide residues were detected in the orange samples. Further, all detected residues did not exceed the MRLs value except propamocarb that exceeded the MRL value by almost 4 times. Gad Alla *et al*, 2015 revealed 17 pesticide residues in 25 orange samples, however, fenitrothion, phenthoate, and profenofos pesticides exceeded the MRLs values. In the same trend, Abdel Latif *et al*.,

2011 stated that from 31 orange samples analyzed, 22 samples were contaminated by 8 pesticide residues. Further, they found that quinalphos was the only pesticide that exceeded the MRL value. Dogheim *et al*., 2002 evaluated 100 orange samples and found 6 pesticide residues, however, dimethoate was the most frequent residue found in the samples, and none of the samples exceeded the MRLs values. Suárez-Jacobo *et al.*, 2017 found 15 pesticide residues in the tested orange samples, however, some orange samples were above MRLs issued by the EU standard. El-Saeid *et al*., 2016 assessed 144 samples of organic and non-organic orange samples, they found that insecticides were detected mostly in the samples then fungicides. On the other hand, methomyl was the violating compound in South African orange and ethion in Egyptian one. Blasco *et al*., 2005 stated that among 116 samples tested, 19 samples contained residues of two or three of the studied pesticides, and no sample exceeded the MRLs values.

To sum up, pesticide residues were found in tested orange samples. Although there is no health risk found, the residues were considered of impact issues. These residues may have retrograde health effects on the environmental components. The proposition solution for this impact issue is that a strict monitoring pesticide residues program must be applied for the orange samples especially the samples that are targeted for export. Further, stakeholders must focus to reduce pesticide use and increase awareness programs to enhance food safety.

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ARABIC SUMMARY

تقدير متبقيات المبيدات في عينات البرتقال بمحافظة اسيوط، مصر

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يعد البرتقال من أهم محاصيل الموالح في مصر، إلا أنه يواجه العديد من المشاكل أهمها متبقيات المبيدات نتيجة للاستخدام غير الواعى للمبيدات. في هذه الدراسة، تم إجراء تقدير وتقييم كمى لمتبقيات المبيدات باستخدام جهاز LC-MS/MS وطريقة QuEChERS لعينات البرتقال في بعض أسواق محافظة أسيوط بمصر (مراكز البداري ومدينة أسيوط ومنفلوط والقوصية وديروط). أظهرت النتائج وجود 14 من متبقيات المبيدات، منهم مبيد واحد فقط تجاوز الحد الأقصى المسموح به في مركزين (البداري والقوصية) (مبيد فلوزيلازول 0.02 و 0.03 ملجم ⁄ كجم). أيضاً وجد أن مبيد البيردابن هوالأكثر شيوعاً لمتبقيات المبيدات المرصودة في 3 مراكز (مدينة أسيوط ومنفلوط وديروط). وجد أيضاً عدم وجود معامل للخطر الصحي لمتبقيات المبيدات المرصودة. وبصفة عامة، يوصى بإجراء برنامج لتحليل متبقيات المبيدات لرصد متبقيات المبيدات في البرتقال للحفاظ على سلامة الغذاء خاصة في صعيد مصر.