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Monitoring of Pesticide Residues in some Cotton Products in Egypt using GC-MS/MS and LC-MS/MS

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ABSTRACT

Nowadays, the worldwide market moves towards the increasing of the domestic supply of organic products so the local markets in Egypt need to be monitored to evaluate the risk of these products in scientific terms. Pesticide residues were determined in some cotton products such as medical cotton, raw cotton, medical gauze, cotton surgical face masks, tissues paper, and diapers were collected from different local markets in Egypt during 2017. Pesticide residues were extracted using modified QuEChERS method followed by liquid and gas chromatography coupled to triple quadrupole tandem mass spectrometry (LC-MS/MS & GC-MS/MS). The target pesticides belong to various chemical groups; most of them are listed in the international standards such as Oeko-Tex standards and the EU ecolabel for textile products as well as the approved recommendations of The Egyptian Agricultural Pesticide Committee (APC-Egypt, 2016). Among the collected cotton product samples, 14 different pesticides were detected in 20 samples in the concentration range from 0.01 to 0.416 mg/kg for the detected pesticides. The obtained results reflected that chlorpyrifos, malathion, profenofos and cypermethrin were the most frequently detected pesticide residues in the cotton product samples. The pesticide frequency percentage were observed in descending order in Chlorpyrifos, Malathion, Profenofos, Cypermethrin, Acetamiprid, Chlorfluazuron, Lufenuron, Cyhalothrin-Lambada, Tricyclazole, Bifenthrin, Flonicamid, Hexachlorobenzene, Methomyl and Piperonyl butoxide with percentages of (38, 10, 10, 8, 6, 6, 6, 4, 4, 2, 2, 2, 2 and 2) respectively. only one raw cotton sample has exceeded the summation of detected pesticide residues.

Key words: Pesticides, QuEChERS, cotton products, Oeko-Tex, LC-MS/MS and GC-MS/MS.

Introduction

Cotton is the most abundant natural polymer with over 25 million tons produced annually. Cotton production, consumption, and trade are the most successful works in the world (Dochia *et al*., 2012). Cotton plays an important role as a major source of revenue (Jody Campiche *et al*., 2016).

According to General Organization of Export and Import Control (GOEIC), Egypt is one of the most important countries for textiles industry of yarns, fabrics, and ready-made garments in the Middle East. GOEIC also reported that 30% of the local employments are working in textiles and Ready-Made Garments (RMG) sector (GAFI, 2014).

About 1326 insects have been recorded in cotton worldwide which make the cotton crop contains more insects than any other crop. Hence, the entomologists find it challenging to fight the

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cotton economic damage that comes from the insects without exceeding the pesticide usage. Approximately 60% of all agrochemicals, being marketed, are insanely applied on cotton fields so that cotton has become one of the most polluted and chemical-intensive agricultural crop in the world (Kranthi and Russell, 2009).

Most of the cotton lint is used in industrial fabrics and medical usage. There are many cotton products in the local markets in Egypt that need to be monitored to check the safety of these products from the pesticides. The most important cotton products are raw cotton, medical cotton, medical gauze, cotton surgical face masks, tissues paper, and diapers (Gordon and Hsieh, 2007; Reddy and Yang, 2009).

Approximately, 90% of the human pesticide poisoning is caused by dermal absorption of pesticides (Van Engelen and Prudhomme de Lodder, 2004) that requires movement of pesticides from the environment through the skin (Baynes and Riviere, 2001; Baynes and Riviere, 2012). Percutaneous absorption of pesticides by pesticide-contaminated cotton products must be noted here because there is a reaction of absorbed pesticides from fabrics to the human skin leading to unhealthy conditions (Baynes and Riviere, 2001; Dix, 2001; Baynes and Riviere, 2012).

Clothes with higher cotton content pick up more pesticides and the amount of transferred pesticide from the contaminated fibers to the skin by mechanical rubbing depends on the pesticide-fabric bonds (Obendorf *et al*., 1994).

In pediatric hospitals in Viet Nam, about 741 cases were detected and 177 infants died because of the infant's hemorrhagic phenomenon due to contamination of infant clothing by talcum powder with the anticoagulant or rodenticide warfarin, added as a perfuming agent (Martin-Bouvyer *et al*., 1983).

In a study on the percutaneous absorption of treated fabrics with carbon-14-labeled dimethylol-dihydroxy ethylene urea to the shaved skin of rabbits where after 48 hours, less than 3% of the concentration of radioactive carbon transferred from the cloth to the rabbit (Hatch *et al*., 1992; Robbins *et al*., 1984). The last accidents and studies were the evidence of the percutaneous absorption of pesticides from cotton products.

Some international organizations such as Oeko-Tex gave a summation of detected pesticides maximum residue limit (MRL) equal to 0.5 mg/kg for cotton products used by babies and 1.0 mg/kg for cotton products used by adults (Oeko-Tex, 2015). Cotton shall not contain more than 0.5 mg/kg for the summation of detected pesticides according to the EU eco-label for textile products user manual commission decision (EU eco-label, 2014).

As the world interest in an environmentally friendly way to produce the organic cotton so the cotton products market need to be monitored to check the truth of these claims by analysis of these cotton products.

The survey in this study covers most of the pesticides listed in the international association for research and testing in the field of textile ecology Oeko-Tex Standard 100 (Oeko-Tex, 2015), the EU eco-label for textile products (EU eco-label, 1999, 2014), and the approved recommendations of Egyptian agricultural pesticide committee (APC-Egypt, 2016).

The aim of the present study is to monitor the presence of pesticide residues, registered by local authorities and commonly used in cotton fields to check their agreement with existing international regulations. The results of the monitoring program were taken into consideration to evaluate the contamination by pesticide residues through the studied cotton products.

Materials and Method

Reagents and Chemicals:

All chemicals were HPLC grade such as (99.8%) toluene, (99.9%) acetonitrile, (99.9%) methanol, (97%) n-hexane, (98-100%) formic acid, (30%) ammonia solution, and (99.8%) glacial acetic acid were purchased from Sigma-Aldrich (USA). QuEChERS Kits 5982-5650 reagent (1) and 5982-5056 reagent (2) were purchased from Agilent Technologies (USA). Deionized water was produced by a mille Q unit (Mille Pore).

A total of 412 pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with purities > 95% including 75 GC- pesticides, 199 LC- pesticides and 138 common

pesticides analyzed by the both techniques. Injection standard solution of 0.1 µg/ml Aldrin in a mixture of n-hexane: acetone (9: 1 v/v) was used for GC-MS/MS injection.

Test samples:

A total of 20 samples of 7 commonly cotton products were selected from different sources from the local markets in Egypt in 2017 which are described in Table 1.

Table 1: Types and number of the collected samples from local markets in Egypt.

Cotton products	Number of samples	Governorates
Raw cotton	6	Cairo, Qaliobia & Giza
Medical cotton	3	Cairo, El Behera & Al Gharbiyah
Medical gauze	1	Cairo
Cotton surgical face masks	1	Cairo
Tissues paper	4	Cairo
Baby diapers	4	Cairo
Adult diapers	1	Cairo

Sample pre-treatment and homogenization:

Pre-treatments were performed to get homogeneous and representative sub-sample to overcome the problematic heterogeneity of pesticides in the samples of cotton products. Representative samples about 100 g were taken and cut into tiny pieces smaller than 5 × 5 cm. The tiny cotton pieces were placed in FRITSCH cutting mill pulverisette 19. The resulting cotton product powder was sampled.

Extraction and cleanup:

Pesticide residues were extracted by using modified citrate buffered QuEChERS method which is the most common widely used method for determination of pesticide residues by LC-MS/MS and GC-MS/MS with 0.01 mg/kg limit of quantitation (LOQ) for most of all detected pesticides (Anastassiades and Lehotay, 2003; Lehotay *et al*., 2005). Validation and development data of the modified QuEChERS method for pesticide residues analysis in cotton is under publication.

A weight of 2.0 g from a milled cotton product into 50.0 ml falcon tube with 10 ml deionized water was shaken vigorously for 1 minute by Geno/Grinder device to wet the cotton sample. A volume of 10 ml of acetonitrile was added and shaken vigorously for 1 minute by Geno/Grinder device. After that reagent (1) was added and then shaken vigorously for 1 minute by Geno/Grinder device. The sample was centrifuged for 5 minutes at 4500 rpm. An aliquot of the supernatant portion was injected directly to LC-MS/MS system for analysis.

The dispersive solid phase extraction (d-SPE) was performed by using reagent (2) where the remaining supernatant was transferred to 15 ml centrifuge tube and shaken vigorously for 1 minute followed by centrifugation at 4500 rpm for 2 minutes. 2 ml from the supernatant was taken into a 100 ml glass flat bottom flask and evaporated by using a rotary evaporator at 280 rpm and 39 °C until drying. The final residue was reconstituted in 2 ml injection standard solution for GC/MS-MS system.

Instrumentation:

LC-MS/MS system:

HPLC (Agilent) 1200 Series instrument coupled to API 4000 Qtrap MS/MS from AB Sciex with electrospray ionization (ESI) interface in the positive mode, source temperature was 400 °C, and ion spray potential was 5500 V.

Separation was performed on an Agilent C18 column ZORBAX Eclipse XDB 4.6 x 150 mm with 5.0 µm particle size. The injection volume was 5.0 µl. As shown in Table 2 a gradient elution program was used at 300 µl/min flow rate, in which one reservoir contained a mobile phase buffer of 10 mM ammonium formate solution in methanol: water (1: 9 v/v) at pH= 4 and the other reservoir contained LC-MS grade Methanol. The run time was 32 minutes.

Table 2: The LC Gradient Elution program.

Time (min)	Mobile Phase Buffer %	Methanol %
0	100	0
13	5	95
21	5	95
28	100	0
32	100	0

GC-MS/MS System:

The analysis was carried out using a 7890A Agilent Gas Chromatography system equipped with a 7000B triple quadrupole mass spectrometer (Agilent Technologies, USA). Chromatographic separations were accomplished using the DB-35MS ultra-inert capillary column (5% biphenyl-95% dimethylsiloxane, 25 m column length x 0.18 mm id x film thickness 0.25 µm) from (Agilent Technologies, USA).

The inlet operating conditions were 1 µl injection volume, 1.3 ml/min the flow rate with GC oven programming temperatures as shown in Table 3 where the samples were injected in a splitless mode and the run time was 25 minutes. The Electron impact (EI) mode was at (+70 eV) with ion source, injector, and quadruple analyzer temperatures were fixed at 300, 250 and 150 °C, respectively.

Table 3: The GC oven programming temperature.

	Rate (°C/min)	Temperature (°C)	Hold time (min)	Run time (min)
Initial		20	1	1
Ramp1	50	150	0	2.6
Ramp2	6	260	0	20.933
Ramp3	20	310	1.567	25

Results and Discussion

Monitoring Results:

A total of 14 pesticides were detected in the 20 surveyed samples by using LC-MS/MS and GC-MS/MS techniques. The detected pesticides were classified as one carbamate (Methomyl), one neonicotinoid (Acetamiprid), one organochlorine (Hexachlorobenzene), one reductase (Tricyclazole), two urea derivatives (Chlorfluazuron and Lufenuron), three Pyrethroid (Bifenthrin, Cyhalothrin-L and Cypermethrin), three organophosphorus (Chlorpyrifos, Malathion and Profenofos) and two unclassified (Flonicamid and Piperonyl butoxide) pesticides.

The concentrations of the detected pesticide residues in raw cotton in 3 different governorates (Cairo, Giza, and Qaliobia) are displayed in Table 4.

As shown in Table 5, a total of 6 different pesticide residues were detected in medical product samples which were unexpected results but with concentrations less than the maximum limits reported by Oeko-Tex standard and EU eco-label user manual. Thus the tested samples of medical cotton products were consistent with the international standards.

The most contaminated cotton product was the raw cotton which is may be due to the absence of industrial treatment processes that have been done on the medical cotton products.

As shown in Figure 1 the frequency percentages of the 14 detected pesticide residues in all analysed samples in descending order were chlorpyrifos (38%), Malathion and Profenofos (10% for each), cypermethrin (8%), Acetamiprid, Chlorfluazuron, and Lufenuron (8% for each) lambda-cyhalothrin and Tricyclazole (4% for each), and Bifenthrin, methomyl, Flonicamid, Hexachlorobenzene and Piperonyl butoxide (2% for each).

Table 4: The results of concentrations of detected pesticides in raw cotton samples.

Cotton Products	Detected pesticides	Concentration (mg/kg)
Raw cotton (Cairo)	Chlorpyrifos	< LOQ
	Bifenthrin	0.040
Raw cotton (Cairo)	Chlorpyrifos	< LOQ
Raw cotton (Giza)	Chlorpyrifos	0.210
	Acetamiprid	0.015
	Profenofos	0.300
	Cyhalothrin-L	0.056
	Cypermethrin	0.242
	Lufenuron	0.010
	Chlorfluazuron	0.088
Raw cotton (Giza)	Chlorpyrifos	0.300
	Acetamiprid	< LOQ
	Profenofos	0.290
	Cyhalothrin-L	0.090
	Cypermethrin	0.416
	Lufenuron	0.050
	Chlorfluazuron	0.043
	Methomyl	0.028
	Tricyclazole	0.016
	Malathion	< LOQ
Raw cotton (Qaliobia)	Chlorpyrifos	0.010
	Acetamiprid	< LOQ
	Profenofos	< LOQ
	Flonicamid	< LOQ
	Cypermethrin	0.010
Raw cotton (Qaliobia)	Chlorpyrifos	0.153
	Profenofos	0.010
	Cypermethrin	0.113
	Lufenuron	0.014
	Chlorfluazuron	0.012
	Tricyclazole	< LOQ
	Malathion	< LOQ

Table 5: The results of concentrations of detected pesticides in medical cotton product samples.

Cotton Products	Detected pesticides	Concentration (mg/kg)
Disposable face mask (Cairo)	Chlorpyrifos	0.010
Medical cotton (Cairo)	Chlorpyrifos	< LOQ
Medical cotton (El Behera)	Chlorpyrifos	0.010
	Piperonyl butoxide	0.010
	Malathion	< LOQ
Medical cotton (Al Gharbiyah)	Chlorpyrifos	< LOQ
Medical Gauzes (Cairo)	Chlorpyrifos	< LOQ
Tissue paper (Cairo)	Chlorpyrifos	0.016
Tissue paper (Cairo)	Chlorpyrifos	0.010
Tissue paper (Cairo)	Chlorpyrifos	< LOQ
Tissue paper (Cairo)	Chlorpyrifos	< LOQ
Baby Diapers (Cairo)	Chlorpyrifos	< LOQ
Baby Diapers (Cairo)	Chlorpyrifos	0.030
	Hexachlorobenzene	< LOQ
	Malathion	< LOQ
Baby Diapers (Cairo)	Chlorpyrifos	0.010
Baby Diapers (Cairo)	Chlorpyrifos	< LOQ
Adult Diapers (Cairo)	Chlorpyrifos	0.010
	Malathion	< LOQ
	Profenofos	< LOQ

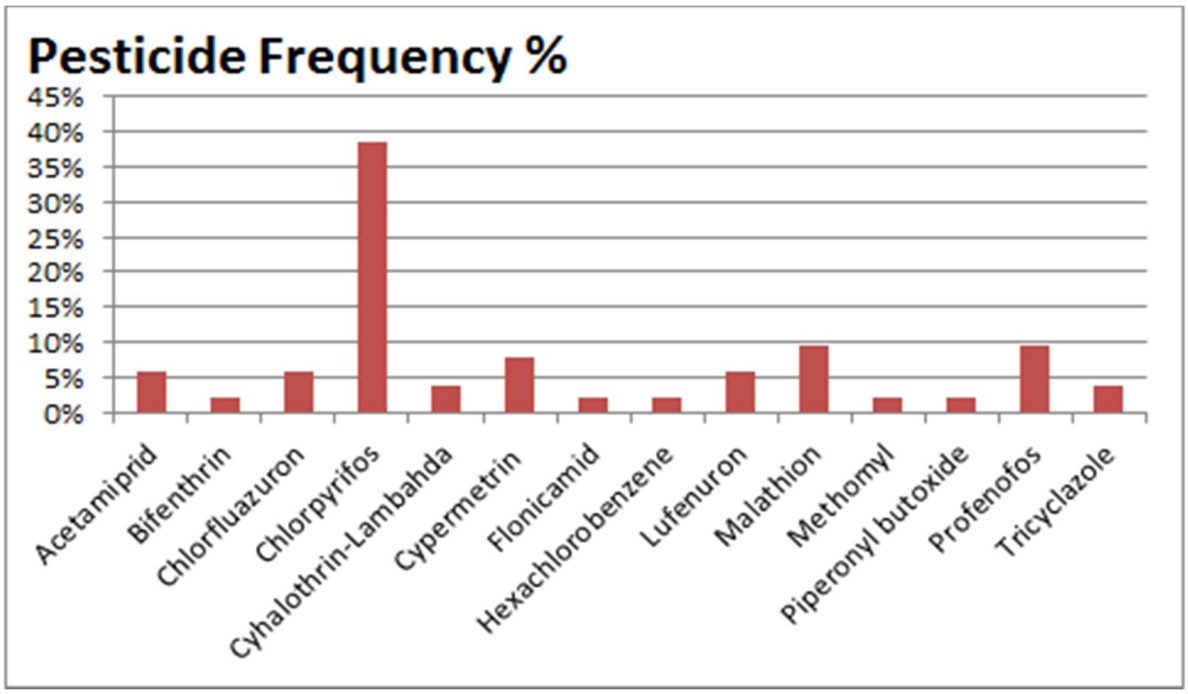

Fig. 1: The frequency percentages of the detected pesticide residues in all samples.

Chlorpyrifos was the most detected pesticide which has a broad spectrum activity for application on cotton plant crops. In the tested samples, 14 pesticide residues were detected in the concentration range of 0.01-0.416 mg/kg. Only one sample (raw cotton sample from Giza) has 1.238 mg/kg summation of pesticide residues concentrations so this sample has exceeded the summation of pesticides maximum residue limit of 1.0 mg/kg according to Oeko-Tex standards.

Internal Quality Control (IQC):

According to ISO 17025, the IQC is an important item in the technical requirements (EN ISO/IEC 17025, 2005). In the routine analysis, the IQC was carried out using spiked blank samples at 0.05 µg/ml were carried out to assess the extraction efficiency. As shown in Tables (7 & 8) the range of recoveries for all analytes varied between 75-116 % which is acceptable range according to SANTE and Eurachem guidelines (Magnusson & Ornemark, 2014; SANTE/11945/2015, 2015/2016).

Table 7: The spiking recovery % for detected pesticide residues by LC-MS/MS.

Compounds	Spiking Recovery %
Chlorpyrifos	84
Acetamiprid	93
Profenofos	86
Cyhalothrin-L	75
Cypermethrin	93
Lufenuron	84
Chlorfluazuron	79
Methomyl	95
Piperonyl butoxide	84
Tricyclazole	95
Flonicamid	94

Table 8: The spiking recovery % for detected pesticide residues by GC-MS/MS.

Compounds	Spiking Recovery %
Bifenthrin	96
Chlorpyrifos	116
Hexachlorobenzene	85
Malathion	106
Cyhalothrin-L	100
Cypermethrin	82
Piperonyl butoxide	99
Profenofos	106

Conclusion

In this study, monitoring of the pesticide residues in cotton products was studied to encourage the confidence and the trust in the Egyptian cotton products in the international market demand and control health risks. It was concluded that Chlorpyrifos is the major detected pesticide residue with high percentages of frequency in some cotton products in Egypt especially in raw cotton. The obtained results from this survey show that only one sample has exceeded the summation of maximum residue pesticides limit, the rest of the samples also contained pesticides but doesn't exceed the international limits.

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