FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (39), No. 3, pp. 525-543, 2019

MICR MONITOR OF CERTAINE PESTICIDE RESIDUES IN GREEN BEANS AND POMEGRANATE IN MINIA GOVERNORATE (EGYPT).

(1)Esraa, A. Mohamed; (1)Gamal, A. Rizk; (2)Monir M. M. Almaz

(1) Plant Protection Dept., Faculty of Agric., Minia Univ.
(2) The Central Agricultural Pesticides, Agric. Research Center

Received: 12 Feb. (2020) Accepted: 15 April (2020)

ABSTRACT

The present study was carried out to investigate a simple monitoring Program. Monitoring study of pesticide residues was carried out in Minia Governorate (Egypt) during October 2018. Forty- two samples of green beans and pomegranates collected from 6 local markets in El-Minya Governorate (i.e. Bani Mazar-Matai –Samalout-Minia-Abu Qurqas-Malawi). Samples were extracted using (QuEChERS) method and analyzed using (LC-MS/MS) and (GC-MS/MS). Results showed that 21.43% of the samples had no detectable pesticide residues. However, 59.57% contained detectable residues without violation and 19% contained residues that exceeded the maximum residues limits (MRL's). Green Beans samples recorded the highest contamination percentages without exceeding of the levels of MRL's (i.e.72.22%), followed by pomegranate (i.e.50%). Data showed that pomegranates recorded the highest violation (i.e.33.33%), while green bean samples free of violation pesticide residues. The highest frequently detected pesticide in all samples were Cypermethrin(13) followed by Imidacloprid(13) carbendazim(11), acetamiprid(8), Cyfluthrin(7),Thiophanate-methyl(7), lambada-cyhalthrin(5), Spirodiclofen(4), chlorpropham(3), Chlorpyrifos(3), Fenpyroximate(2) and Methomyl(2), and the synergist pipernyl butoxide which detected three times. While the lowest frequently detected pesticides, which detected only one time in these samples were Carbofuran, carbofuran3-hydroxy, Diazinon, Dimethoate, Lufenuron, Methoxyfenozide, Omethoate, Profenofos and Tetramethrin. The violation pesticides in pomegranate were seven

i.e. Acetamiprid, Cyfluthrin, Chlorpyrifos, Dimethoate, Omethoate, Methomyl and Thiophanate- methyl. The most contaminated green beans samples and pomegranate were found in Minia and Matay market followed by Abu Qarqas then Bani Mazar followed by Malawi and Samalut and farm in Malawi. The results also showed that fifteen samples contained residues of one pesticide and two samples contained residues of eight pesticides.

Key words: Monitoring, Pesticide residues, Pomegranate, Green Beans.

INTRODUCTION:

No doubt, that the use of pesticides has resulted in increasing agricultural production. Worldwide pesticides are used to protect agriculture products before and after harvest from infestation by pests and plant diseases. A possible consequence of their use may be the presence of pesticide residues in the treated products, but some persistent pesticide residues have great potential of adverse impact on the environment and human health. Application of pesticides in modern agriculture has boosted farm productivity (Krol. *et al.,* 2000). Vegetables and fruits are commonly used everywhere to meet the requirement of balance diet (Bempah and Donkor, 2011). Vegetables and fruits play an important role in human nutrition and health (Mebdoua, 2018). Pesticides contamination is a main international of trade problem (Beena and Kathpal, 2009). Several pesticides are toxic substances and can persistent in the environment for a long time. Therefore, it is necessary to control the application of pesticides on crops (Freidberg, 2003).

However, levels of pesticides should be controlled at optimum point due to their relative toxicity to the environment and human health (Jiang. *et al.,* 2009).

The misuse or high dosage of pesticides leads to the contamination of pesticides in their agricultural products which may be of health risk to the consumer. Thus, the monitoring of pesticide residues in vegetables and fruits have become ever more essential requirement for consumers, producers and institutions concerned with standards and quality control management (Beena. *et al.,* 2006). A lot of studies had been conducted to determine the pesticides residues in fruits and vegetables (i.e. Kocourek, *et al.,* 1998, Zambonin *et al.,* 2004, Gambacorta, *et al.,* 2005, Rissato, *et al.,* 2007, Zhang, *et al.,* 2007 Thabet, *et al.,* 2016 and Stachniuk *et al.,* 2017).

The aim of this study was to determine the level of some pesticide residues in green beans and pomegranate, available on the local markets to give a simple monitoring program which will appear true picture of contamination with pesticide in green bean and pomegranate in Minia Governorate districts (Egypt) i.e. Bani Mazar-Matai –Samalout-Minia-Abu Qurqas-Malawi).

MATERIALS AND METHODS

Sampling:

In this study 42 samples of green beans and pomegranate were collected from six local markets in El-Minia Governorate districts (Egypt) (i.e. Bani Mazar- Matai –Samalout-Minya-Abu Qurqas-Malawi) during October 2018.For residue analysis ,2 kg of each commodity was prepared according to Codex guidelines (2010) .The generally recommended method of sampling was used to obtain a representative part of the material to be analyzed. Samples were analyzed immediately upon their arrival at the laboratory, or they were stored at -5°C for no longer than 2 days before analysis.

PESTICIDE RESIDUES ANALYSIS:

The standard method European Committee for Standardization/Technical Committee 275 (2007) for foods of plant origin: pr EN 15662 (QuEChERS) was followed. The determination of residues carried out using GC-MS/MS and LC-MS/MS after acetonitrile extraction/partitioning and cleanup by dispersive SPE.

The homogeneous sample is extracted in frozen condition with the help of acetonitrile. Samples with low water content (< 80%) require the addition of water before the initial extraction to get a total of approximately 10g of water. After addition of magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to 5.5). The mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is cleaned-up by dispersive solid phase extraction (D-SPE) employing bulk sorbent as well as magnesium sulfate for the removal of residual water. Following cleanup with amino-sorbents (e.g. primary secondary amine sorbent, PSA) extracts are acidified by adding a small amount of formic acid, to improve the storage stability of certain base-sensitive pesticides. The final extract can be directly employed for GC- and LC-based determinative analysis. Quantification is performed using an internal standard, which is added directly before injection in GC-MSD system. The method validated 78 compounds using LC-MS/MS and 26 compounds using GC-MS/MS. The detection and confirmation of pesticide residues in the samples was made using both GC-MS/MS and LC-MS/MS.

Quality Assurance procedures:

All analytical methods and instruments were carefully validated as a part of the laboratory quality assurance system and were audited and accredited by the Center of Metrology and Accreditation Finnish. (Accreditation Service (FINAS) ISO/IEC Guide 25). The criteria of quality assurance were followed to determine the performance of the standard method. The average recoveries tests on different types of pesticides at different concentration levels varied between 70-120%. The reproducibility expressed as relative standard deviation was less than 25%. The limit of quantification started at 0.01 mg/kg and up depending on the pesticide type and detected module.

The measurement uncertainty expressed as expanded uncertainty and in terms of relative standard deviation (at 95% confidence level) is lower than the default value set by EU (± 50%). Blank samples were fortified with the pesticides mixture and analyzed as a normal sample with set of samples. The results were recorded on control charts. Repeated analysis of old samples was regularly carried out to control reproducibility.

Apparatus:

(a) LC-MS/MS System

Agilent 1200 series liquid chromatography system equipped with Applied Biosystems (API 4000 Qtrape) tandem mass spectrometers with electrospray ionization (ESI) interface. Separation was performed on a C18 column ZORBAX Eclipse XDBC18 4.6 mm x 150 mm, 5 µm particle sizes. The injection volume was 25 µl. A gradient elution program was at 0.3 ml/min flow rate, in which one reservoir contained 10 mM ammonium formate solution in MeOH:H2O (1:9, v/v) and the other contained methanol. The ESI source was used in the positive mode, and Nitrogen was used as nebulizer gas, curtain gas, heater gas and collision gas according to manufacturer's settings; source temperature was 300oC, ion spray potential 5500 V, decluster potential and collision energy were optimized using a Harvard apparatus syringe pump. The Multiple Reaction Monitoring Mode (MRM) was used in which one MRM was used for quantification and other was used for confirmation.

(b) GC-MS/MS System

Agilent Gas Chromatograph 7980A equipped with tandem mass spectrometer 7000B Quadrupole, EI source was used to perform analysis by using HP-5MS 5% phenyl methyl siloxane capillary column (30 m length x 0.25 mm id x 0. 25 µm film thickness). Samples were injected in a splitless mode and helium was used as carrier gas (1 ml/min). Injector temperature was 250°C transfer line temperature was 285°C, ion source temperature was 280°C and quadrupole temperature was 150°C. The GC oven temperature was programmed to initially held at 70°C for 2 min then increased to 150°C at 25°C/min (held for 0 min), and raised to 200°C at the rate of 3°C/min (held for 0 min), then went up from 200 to 280°C at 8°C/min (held for 10 min). This resulted in a total run time of 42 min and complete separation of all the analysts.

Reagents:

Solvent and chemicals described in the standard method CEN 275, 2007.

Pesticides reference standards:

All reference materials are certified provided by Dr. EhrenstorferGmbh, Gogginger Str. 78 D-8900 Augoburg.

Rate of recovery: -

Table (1) and (2) illustrate Limit of Quantification (LOQ), Recovery% and Coefficient Of Variation (CV) for 104 monitoring pesticide residues and one synergist in green beans and pomegranate, respectively.

RESULTS AND DISCUSSION:

Monitoring results:

The detected pesticides, minimum, maximum, mean detected levels, numbers and percentages of violated and free samples in green bean and pomegranate are shown in Table (3) and Fig (1). A total of 42 samples of Green Bean and Pomegranate were analyzed. Overall, 21.43% of the samples had no detectable pesticide residues and the other 78.57% contained detectable residues. Also, results showed that 59.57% of contaminated samples contain residues at level lower than the MRLs and 19% had residues above the permissible limits.

Green bean Samples:

A total of 18 samples of green beans were subjected to analysis. Data represents in table (3) and Figure (1) showed that 27.78% of green bean samples were free from any detectable pesticide residues. However, 72.22% of samples contained detectable levels of pesticides residues, but without exceeding of MRL established for each pesticide. Results showed that the highest frequently detected pesticides was Carbendazim followed by Chlorpropham, Lambda-cyhalothrin and the synergist piperonyl botoxide. The lowest frequently detected pesticides, which detected only one time, were Imidacloprid, Methoxyfenozide, Profenofos, Thiophanate-methyl and Tetramethrin.

Pomegranate Samples:

A total of 24 samples of pomegranate were subjected to analysis. Data in table (3) and Figure (1) showed that 16.67% of all samples were free from any detectable pesticide residues. However, 50% of samples contained detectable levels of pesticides residues, but without exceeding of MRL established for each pesticide. Results showed that 33.33% of samples contained residues at levels above their established MRL's. The violated pesticides were Acetamiprid, Cyfluthrin, Chlorpyrifos, Thiophanate-methyl, Methomyl, Dimethoate, Omethoate. Also, data showed that: The highest frequently detected pesticides was Cypermethrin followed by Imidacloprid, Acetamiprid, Cyfluthrin, Carbendazim, Thiophanate-methyl, Spirodiclofen, Lambda-Cyhalothrin, Chlorpyrifos, Methomyl and Fenpyroximate. The lowest frequently detected pesticides, which detected only one time were Carbofuran, Carbofuran3-hydroxy, Chlorpyrifos, Diazinon, Dimethoate, Lufenuron and Omethoate.

Table (1): Limit of Quantification (LOQ), Recovery % and Coefficient of Variation (CV) for 104 Monitored pesticides in green beans.

S.N	Pesticides	LOQ	Spiking Level							
			0.01mg/kg		0.05mg/kg		0.1mg/kg		Mean	
			REC%	CV%	REC%	CV%	REC%	CV%	REC%	CV%
1	Abamectin	0.01	74%	20%	104%	11%	68%	13%	82%	15%
2	Acetamiprid	0.01	91%	12%	92%	4%	78%	3%	87%	6%
3	alpha-HCH	0.05	86%	8%	104%	7%	107%	8%	99%	8%
4	Atrazine	0.01	91%	15%	92%	4%	80%	3%	88%	7%
5	Atraton	0.05	75%	3%	115%	3%	94%	13%	95%	6%
6	Azoxystrobin	0.01	98%	14%	96%	4%	78%	2%	91%	7%
7	Benalaxyl	0.01	95%	14%	93%	3%	76%	4%	88%	7%
8	beta-HCH	0.05	71%	9%	87%	6%	80%	13%	79%	9%
9	Bifenthrin	0.01	71%	6%	94%	14%	73%	9%	79%	10%
10	Biphenyl	0.01	70%	22%	90%	3%	74%	10%	78%	12%
11	Boscalid	0.01	77%	6%	92%	9%	94%	5%	88%	11%
12	Bromacil	0.01	85%	15%	102%	7%	93%	6%	94%	12%
13	Bromopropylate	0.01	82%	6%	113%	3%	114%	8%	103%	6%
14	Bupirimate	0.01	85%	11%	93%	4%	75%	4%	84%	6%
15	Cadusafos	0.01	78%	6%	104%	5%	102%	12%	95%	8%
16	Captan	0.05	77%	20%	86%	3%	87%	4%	83%	9%
17	Carbaryl	0.01	93%	13%	93%	2%	79%	2%	88%	6%
18	Carbendazim	0.01	89%	11%	90%	3%	74%	3%	84%	6%
19	Carbofuran	0.01	89%	13%	101%	13%	99%	11%	96%	12%
20	Carbofuran 3-hydroxy	0.01	89%	13%	101%	13%	99%	11%	96%	12%
21	Chlorfenapyr	0.01	73%	7%	113%	3%	111%	8%	99%	6%
22	Chlofluazuron	0.01	77%	20%	113%	16%	98%	7%	96%	14%
23	Chlorothalonil	0.05	82%	7%	79%	12%	66%	10%	76%	11%
24	Chlorpropham	0.05	87%	12%	96%	9%	98%	9%	94%	6%
25	Chlorpyrifos	0.01	91%	9%	93%	8%	97%	3%	94%	7%
26	Cyfluthrin	0.01	104%	7%	116%	5%	94%	11%	105%	11%
27	Cypermethrin	0.01	116%	26%	111%	22%	85%	15%	104%	21%
28	Cyproconazole	0.01	78%	16%	89%	7%	85%	5%	84%	9%
29	Cyprodinil	0.01	80%	10%	89%	3%	68%	4%	79%	6%
30	Cyromazine	0.01	81%	23%	73%	5%	81%	3%	78%	11%
31	delta-HCH	0.05	81%	19%	90%	5%	98%	5%	90%	10%
32	Deltamethrin	0.01	100%	18%	93%	13%	84%	9%	92%	15%
33	Diafenthiuron	0.01	118%	9%	111%	6%	118%	3%	116%	7%
34	Diazinon	0.01	96%	15%	94%	7%	82%	4%	91%	9%
35	Dicofol	0.01	79%	16%	67%	22%	96%	3%	81%	14%
36	Difenoconazole	0.01	86%	17%	84%	7%	74%	4%	81%	9%
37	Dimethoate	0.01	96%	14%	91%	4%	77%	3%	88%	7%
38	Dimethomorph	0.01	99%	13%	94%	4%	89%	8%	94%	8%
39	Diniconazole	0.01	92%	14%	90%	9%	80%	7%	87%	10%
40	Emamectin	0.01	74%	20%	104%	11%	78%	13%	85%	15%
41	Ethion	0.01	76%	14%	79%	8%	72%	4%	76%	9%
42	Ethirimol	0.01	86%	16%	92%	6%	81%	3%	86%	8%
43	Famoxadone	0.01	78%	14%	79%	6%	80%	6%	79%	9%
44	Fenarimol	0.01	78%	14%	90%	5%	77%	4%	82%	8%
45	Fenhexamid	0.01	88%	7%	88%	7%	85%	5%	87%	6%
46	Fenitrothion	0.01	86%	13%	107%	15%	88%	16%	94%	15%
47	Fenpropathrin	0.01	87%	9%	111%	5%	98%	13%	99%	9%
48	Fenpyroximate	0.01	82%	4%	100%	6%	91%	2%	91%	9%
49	Fludioxonil	0.01	84%	2%	85%	5%	96%	6%	88%	5%
50	Flusilazole	0.01	92%	14%	92%	6%	75%	4%	86%	8%
51	Flutolanil	0.01	94%	12%	99%	4%	76%	3%	90%	6%
52	Gamma-HCH	0.05	85%	9%	93%	4%	115%	7%	98%	7%
53	Heptachlor	0.05	107%	6%	91%	23%	96%	14%	93%	14%
54	Hexaconazole	0.01	97%	16%	91%	4%	78%	4%	89%	8%

Table (1): continued

55	Hexythiozox	0.01	89%	8%	96%	6%	98%	4%	94%	7%
56	Imazalil	0.01	113%	20%	98%	4%	74%	3%	95%	9%
57	Imidacloprid	0.01	85%	16%	93%	5%	85%	4%	88%	8%
58	Indoxacarb	0.01	70%	22%	90%	3%	74%	10%	78%	12%
59	Iprodione	0.01	89%	3%	113%	7%	89%	12%	97%	7%
60	Kresoxim-methyl	0.01	79%	4%	101%	2%	75%	5%	85%	4%
61	Gamma-Cyhalothrin	0.01	109%	8%	111%	6%	104%	4%	106%	6%
62	Lambda-Cyhalothrin	0.01	88%	14%	92%	4%	86%	3%	89%	7%
63	Lufenuron	0.01	86%	18%	85%	16%	84%	14%	85%	16%
64	Malaoxon	0.01	89%	15%	93%	5%	87%	4%	90%	8%
65	Malathion	0.01	95%	18%	97%	3%	81%	6%	91%	9%
66	Metalaxyl	0.01	104%	6%	91%	8%	98%	4%	98%	6%
67	Methamidophos	0.01	73%	18%	78%	3%	77%	2%	76%	8%
68	Methomyl	0.01	95%	20%	90%	5%	89%	3%	91%	9%
69	Methoxyfenozide	0.01	82%	20%	102%	11%	83%	4%	89%	12%
70	Myclobutanil	0.01	92%	17%	95%	5%	85%	4%	91%	9%
71	Omethoate	0.01	82%	20%	81%	5%	84%	2%	82%	9%
72	Ortho-Phenyl Phenol(OPP)	0.05	76%	4%	109%	3%	106%	10%	97%	6%
73	Oxamyl	0.01	99%	16%	89%	5%	80%	4%	89%	8%
74	p.p-DDD	0.01	81%	3%	98%	6%	99%	10%	93%	6%
75	p.p-DDE	0.01	75%	7%	101%	3%	102%	11%	93%	7%
76	P,p- DDT	0.05	98%	11%	101%	8%	107%	6%	102%	8%
77	Penconazole	0.01	78%	16%	89%	3%	85%	5%	84%	8%
78	Permethrin	0.01	94%	5%	110%	6%	109%	8%	104%	6%
79	Phenthoate	0.01	87%	19%	91%	4%	86%	5%	88%	9%
80	Phosalone	0.01	77%	19%	82%	3%	80%	9%	80%	10%
81	Piperonyl butoxide	0.01	78%	20%	90%	14%	85%	11%	84%	15%
82	Procymidone	0.01	102%	4%	108%	5%	98%	7%	102%	5%
83	Profenofos	0.01	75%	19%	85%	4%	82%	5%	81%	9%
84	Promecarb	0.01	88%	20%	95%	3%	87%	4%	90%	9%
85	Propamocarb	0.01	81%	20%	73%	5%	80%	3%	78%	9%
86	Propargite	0.01	87%	6%	96%	9%	97%	4%	93%	6%
87	Propiconazole	0.01	78%	20%	89%	3%	86%	4%	84%	9%
88	Pyraclostrobin	0.01	91%	6%	110%	7%	99%	3%	100%	10%
89	Pyridaben	0.01	86%	7%	82%	9%	77%	5%	82%	7%
90	Pyriproxyfen	0.01	76%	4%	98%	8%	99%	3%	91%	13%
91	Spinosad	0.01	78 %	17%	84%	5%	71%	13%	78%	12%
92	Spirodiclofen	0.01	87 %	19%	91%	4%	86%	5%	88%	9%
93	Sulfur	0.05	107%	20%	114%	12%	101%	13%	107%	15%
94	Tetraconazole	0.01	92%	16%	93%	3%	87%	5%	91%	8%
95	Tetramethrin	0.01	92 %	18%	94%	4%	88%	6%	91%	9%
96	Thiabendazole	0.01	86%	16%	92%	6%	81%	3%	86%	8%
97	Thiacloprid	0.01	84%	16%	90%	4%	82%	3%	85%	8%
98	Thiamethoxam	0.01	87%	20%	84%	4%	91%	4%	87%	9%
99	Thiobencarb	0.01	77%	20%	86%	3%	87%	4%	83%	9%
100	Thiophanate-methyl	0.01	74%	15%	83%	19%	88%	11%	82%	15%
101	Tolylfluanid	0.01	76%	20%	89%	6%	80%	9%	82%	12%
102	Triadimenol	0.01	97%	19%	87%	8%	80%	4%	88%	10%
103	Trifloxystrobin	0.01	73%	16%	86%	12%	85%	11%	81%	13%
104	Triticonazole	0.01	85%	19%	89%	11%	84%	14%	86%	15%

Table (2): Limit of Quantification (LOQ), Recovery % and Coefficient of Variation (CV) for 104 Monitored pesticides in Pomegranate.

S.N	Pesticides	LOQ	Spiking Level							
			0.01mg/kg		0.05mg/kg		0.1mg/kg		Mean	
			REC%	CV%	REC%	CV%	REC%	CV%	REC%	CV%
1	Abamectin	0.01	94%	13%	76%	9%	85%	17%	85%	13%
2	Acetamiprid	0.01	97%	5%	95%	7%	83%	3%	92%	5%
3	alpha-HCH	0.05	79%	12%	77%	11%	96%	8%	84%	10%
4	Atrazine	0.01	88%	8%	98%	13%	87%	6%	91%	9%
5	Atraton	0.05	78%	14%	88%	19%	103%	15%	90%	16%
6	Azoxystrobin	0.01	100%	6%	98%	9%	89%	4%	96%	6%
7	Benalaxyl	0.01	98%	7%	107%	9%	79%	4%	95%	7%
8	beta-HCH	0.05	93%	10%	97%	12%	101%	14%	97%	12%
9	Bifenthrin	0.01	71%	19%	86%	20%	109%	5%	89%	15%
10	Biphenyl	0.01	86%	6%	97%	15%	95%	11%	93%	11%
11	Boscalid	0.01	92%	11%	93%	6%	116%	20%	100%	12%
12	Bromacil	0.01	86%	19%	103%	17%	110%	14%	99%	16%
13	Bromopropylate	0.01	88%	8%	100%	14%	103%	16%	97%	12%
14	Bupirimate	0.01	94%	8%	93%	5%	87%	3%	91%	5%
15	Cadusafos	0.01	88%	5%	100%	11%	99%	7%	96%	8%
16	Captan	0.05	87%	8%	101%	10%	83%	5%	90%	8%
17	Carbaryl	0.01	88%	3%	99%	10%	91%	5%	93%	6%
18	Carbendazim	0.01	87%	10%	85%	8%	74%	4%	82%	7%
19	Carbofuran	0.01	88%	8%	87%	6%	70%	3%	82%	6%
20	Carbofuran 3-hydroxy	0.01	89%	6%	86%	5%	73%	2%	83%	4%
21	Chlorfenapyr	0.01	76%	12%	87%	16%	101%	18%	88%	15%
22	Chlofluazuron	0.01	82%	10%	84%	11%	88%	14%	84%	11%
23	Chlorothalonil	0.05	85%	6%	94%	9%	113%	11%	97%	9%
24	Chlorpropham	0.05	87%	14%	73%	9%	95%	16%	85%	13%
25	Chlorpyrifos	0.01	86%	10%	82%	7%	79%	5%	82%	7%
26	Cyfluthrin	0.01	103%	16%	94%	11%	104%	7%	100%	11%
27	Cypermethrin	0.01	97%	4%	75%	6%	85%	8%	86%	6%
28	Cyproconazole	0.01	91%	5%	92%	8%	89%	4%	91%	5%
29	Cyprodinil	0.01	89%	4%	88%	3%	80%	2%	86%	3%
30	Cyromazine	0.01	85%	13%	76%	7%	82%	11%	81%	10%
31	delta-HCH	0.05	98%	12%	79%	11%	99%	10%	92%	11%
32	Deltamethrin	0.01	84%	8%	89%	13%	85%	9%	86%	10%
33	Diafenthiuron	0.01	86%	6%	87%	8%	88%	11%	87%	8%
34	Diazinon	0.01	86%	4%	108%	7%	81%	3%	92%	5%
35	Dicofol	0.01	93%	10%	84%	18%	97%	16%	91%	15%
36	Difenoconazole	0.01	90%	5%	98%	7%	76%	2%	88%	5%
37	Dimethoate	0.01	96%	12%	94%	9%	86%	7%	92%	9%
38	Dimethomorph	0.01	90%	5%	95%	10%	88%	4%	91%	6%
39	Diniconazole	0.01	88%	6%	92%	7%	80%	3%	86%	5%
40	Emamectin	0.01	94%	13%	76%	5%	85%	7%	85%	8%
41	Ethion	0.01	86%	5%	89%	6%	80%	4%	85%	5%
42	Ethirimol	0.01	79%	7%	101%	11%	75%	5%	85%	7%
43	Famoxadone	0.01	99%	10%	91%	7%	84%	4%	91%	7%
44	Fenarimol	0.01	94%	13%	93%	10%	87%	5%	91%	9%
45	Fenhexamid	0.01	93%	8%	98%	11%	86%	4%	92%	8%
46	Fenitrothion	0.01	110%	13%	72%	4%	94%	11%	92%	9%
47	Fenpropathrin	0.01	86%	19%	72%	4%	102%	20%	86%	14%
48	Fenpyroximate	0.01	78%	11%	70%	7%	76%	9%	74%	9%
49	Fludioxonil	0.01	86%	6%	97%	15%	95%	16%	92%	12%
50	Flusilazole	0.01	89%	5%	97%	9%	87%	4%	91%	6%
51	Flutolanil	0.01	89%	4%	98%	9%	80%	2%	89%	5%
52	Gamma-HCH	0.05	77%	7%	86%	19%	84%	10%	82%	12%
53	Heptachlor	0.05	96%	11%	70%	7%	97%	14%	88%	11%
54	Hexaconazole	0.01	91%	11%	90%	9%	79%	4%	87%	8%

Table (2): continued

55	Hexythiozox	0.01	88%	9%	85%	8%	72%	4%	82%	7%
56	Imazalil	0.01	71%	11%	110%	18%	94%	5%	92%	11%
57	Imidacloprid	0.01	97%	8%	96%	5%	80%	9%	91%	7%
58	Indoxacarb	0.01	90%	6%	92%	9%	95%	13%	92%	9%
59	Iprodione	0.01	89%	3%	110%	6%	93%	11%	97%	12%
60	Kresoxim-methyl	0.01	89%	8%	93%	3%	84%	2%	88%	4%
61	Gamma-Cyhalothrin	0.01	119%	18%	81%	14%	111%	15%	103%	16%
62	Lambda-Cyhalothrin	0.01	89%	5%	92%	7%	82%	3%	87%	5%
63	Lufenuron	0.01	83%	11%	85%	16%	81%	10%	83%	12%
64	Malaoxon	0.01	95%	5%	100%	7%	87%	3%	94%	5%
65	Malathion	0.01	92%	4%	105%	9%	91%	6%	96%	6%
66	Metalaxyl	0.01	89%	8%	91%	17%	112%	20%	97%	15%
67	Methamidophos	0.01	96%	18%	83%	7%	76%	5%	85%	10%
68	Methomyl	0.01	90%	7%	92%	4%	82%	5%	88%	5%
69	Methoxyfenozide	0.01	92%	5%	97%	9%	80%	3%	90%	6%
70	Myclobutanil	0.01	94%	5%	95%	7%	88%	3%	92%	5%
71	Omethoate	0.01	85%	4%	94%	5%	76%	3%	85%	4%
72	Ortho-Phenyl Phenol(OPP)	0.05	83%	4%	97%	15%	94%	8%	91%	9%
73	Oxamyl	0.01	96%	13%	95%	11%	85%	8%	92%	11%
74	p.p-DDD	0.01	79%	4%	87%	11%	96%	13%	87%	9%
75	p.p-DDE	0.01	83%	15%	84%	12%	98%	17%	88%	15%
76	P,p- DDT	0.05	81%	12%	89%	14%	90%	17%	87%	14%
77	Penconazole	0.01	91%	5%	92%	8%	89%	3%	91%	5%
78	Permethrin	0.01	85%	12%	78%	7%	115%	16%	93%	12%
79	Phenthoate	0.01	90%	4%	95%	10%	86%	2%	90%	5%
80	Phosalone	0.01	93%	5%	95%	10%	90%	2%	92%	6%
81	Piperonyl butoxide	0.01	92%	9%	95%	11%	78%	2%	88%	7%
82	Procymidone	0.01	81%	3%	100%	13%	96%	8%	92%	8%
83	Profenofos	0.01	103%	15%	98%	13%	76%	11%	92%	13%
84	Promecarb	0.01	98%	6%	97%	5%	82%	3%	92%	5%
85	Propamocarb	0.01	82%	4%	88%	7%	71%	3%	80%	5%
86	Propargite	0.01	85%	13%	76%	7%	82%	11%	81%	10%
87	Propiconazole	0.01	95%	9%	92%	6%	83%	3%	90%	6%
88	Pyraclostrobin	0.01	94%	9%	95%	10%	92%	8%	93%	9%
89	Pyridaben	0.01	81%	11%	85%	12%	87%	13%	84%	12%
90	Pyriproxyfen	0.01	84%	14%	79%	5%	81%	12%	81%	10%
91	Spinosad	0.01	96%	12%	88%	6%	83%	4%	89%	7%
92	Spirodiclofen	0.01	88%	4%	87%	6%	89%	7%	88%	6%
93	Sulfur	0.05	85%	13%	87%	16%	103%	17%	92%	15%
94	Tetraconazole	0.01	97%	7%	96%	10%	83%	2%	92%	6%
95	Tetramethrin	0.01	92%	6%	89%	4%	80%	3%	87%	4%
96	Thiabendazole	0.01	79%	5%	101%	11%	75%	4%	85%	7%
97	Thiacloprid	0.01	93%	5%	96%	6%	84%	4%	91%	5%
98	Thiamethoxam	0.01	94%	6%	98%	8%	78%	4%	90%	6%
99	Thiobencarb	0.01	87%	8%	101%	10%	83%	3%	90%	7%
100	Thiophanate-methyl	0.01	80%	5%	99%	13%	82%	8%	87%	9%
101	Tolylfluanid	0.01	88%	4%	95%	13%	90%	6%	91%	8%
102	Triadimenol	0.01	87%	3%	98%	13%	88%	7%	91%	7%
103	Trifloxystrobin	0.01	93%	5%	94%	8%	89%	2%	92%	5%
104	Triticonazole	0.01	89%	5%	92%	7%	83%	3%	88%	5%

Table 3: The Number of analyzed Green Beans and Pomegranate samples, contamination and frequencies of pesticides residues as well as the levels of residues and their Violations.

Crop name	Analyzed samples	Total Contaminated		Detected Pesticides	Frequency		Pesticides Level			MRL	The Violated samples		Total Violated samples	
		No.	%		No	%	Min	Max	Median		No.	%	No.	%
Green bean	18	13	72.22%	Carbendazim	5	27.78	0.006	0.04	0.023	0.2	0	0.00	0	0.00
				Chlorpropham	3	16.67	0.006	0.01	0.008	0.01	0	0.00		
				Imidacloprid	1	5.56	0.01	0.01	0.01	2	0	0.00		
				Lambda-Cyhalothrin	2	11.11	0.006	0.006	0.006	0.2	0	0.00		
				Methoxyfenozide	2	5.56	0.006	0.006	0.006	2	0	0.00		
				Piperonyl butoxide	3	16.67	0.006	0.006	0.006	No MRL	0	0.00		
				Profenofos	1	5.56	0.01	0.01	0.01	0.01	0	0.00		
				Thiophanate-methyl	1	5.56	0.06	0.06	0.06	0.1	0	0.00		
				Tetramethrin	1	5.56	0.006	0.006	0.006	0.01	0	0.00		
Pomegranate	24	12	50%	Acetamiprid	8	33.33	0.006	0.04	0.013	0.01	4	16.67	8	33.33%
				Carbendazim	6	25	0.01	0.1	0.025	0.1	0	0.00		
				Carbofuran	1	4.16	0.006	0.006	0.006	0.01	0	0.00		
				Carbofuran 3-hydroxy	1	4.16	0.006	0.006	0.006	0.01	0	0.00		
				Chlorpyrifos	3	12.5	0.01	0.07	0.06	0.05	2	8.33		
				Cyfluthrin	7	29.16	0.01	0.06	0.02	0.02	3	12.5		
				Cypermethrin	13	54.16	0.01	0.03	0.02	0.05	0	0.00		
				Diazinon	1	4.16	0.006	0.006	0.006	0.01	0	0.00		
				Dimethoate	1	4.16	0.04	0.04	0.04	0.01	1	4.16		
				Fenpyroximate	2	8.33	0.006	0.006	0.006	0.01	0	0.00		
				Imidacloprid	12	50	0.006	0.15	0.025	1	0	0.00		
				Lambda-Cyhalothrin	3	12.5	0.006	0.01	0.01	0.02	0	0.00		
				Lufenuron	1	4.16	0.006	0.006	0.006	0.01	0	0.00		
				Methomyl	2	8.33	0.01	0.05	0.03	0.01	1	4.16		
				Omethoate	1	4.16	0.04	0.04	0.04	0.01	1	4.16		
				Spirodiclofen	4	16.67	0.006	0.01	0.008	0.02	0	0.00		
				Thiophanate-methyl	6	25	0.02	0.18	0.05	0.1	1	4.16		

Figure (2) showed that, 21 pesticides and one synergist have been identified as detectable residues in all analyzed samples collected. The highest frequently detected compounds were Cypermethrin and imidacloprid which were detected in 13samples, followed by carbendazim(11samples), acetamiprid(8samples), Cyfluthrin and Thiophanate-methyl(7samples), Lambda-cyhalthrin (5samples), while each of chlorpropham, Chlorpyrifos and the synergist (piperonyl butoxide) were detected in (3samples). Methomyl and Fenpyroximate in (2samples). However, the lower frequently detected pesticides ,which detected only in one sample were Carbofuran, Carbofuran 3-hydroxy, Diazinon, Dimethoate, Lufenuron, Methoxyfenozide, Omethoate, Profenofos and Tetramethrin .

The main pesticides violated in Pomegranate collected were Acetamiprid, Cyfluthrin, Chlorpyrifos, Dimethoate, Omethoate, Methomyl and Thiophanate - methyl.

Table (4) and figure (3) showed demonstrates the situation of calculated contamination and the breakdown of the total number of contaminated samples. Non-Contaminated samples percentage were 21.43 %. The percentage of samples contained one pesticide residues were 35.71 % and which contained two pesticide residues were 11.9%, while the percentage of samples contained three or more pesticide residues were 30.95% .

Table:(4) Percentages of All analyzed samples contain one, two, three or more pesticides residues.

Total no of samples	Non-Contaminated Samples percentages	Percentage of samples contained one pesticides residue	Percentage of samples contained two pesticides residues	Percentage of samples contained three or more pesticides residues
42	21.43%	35.71%	11.9%	30.95%

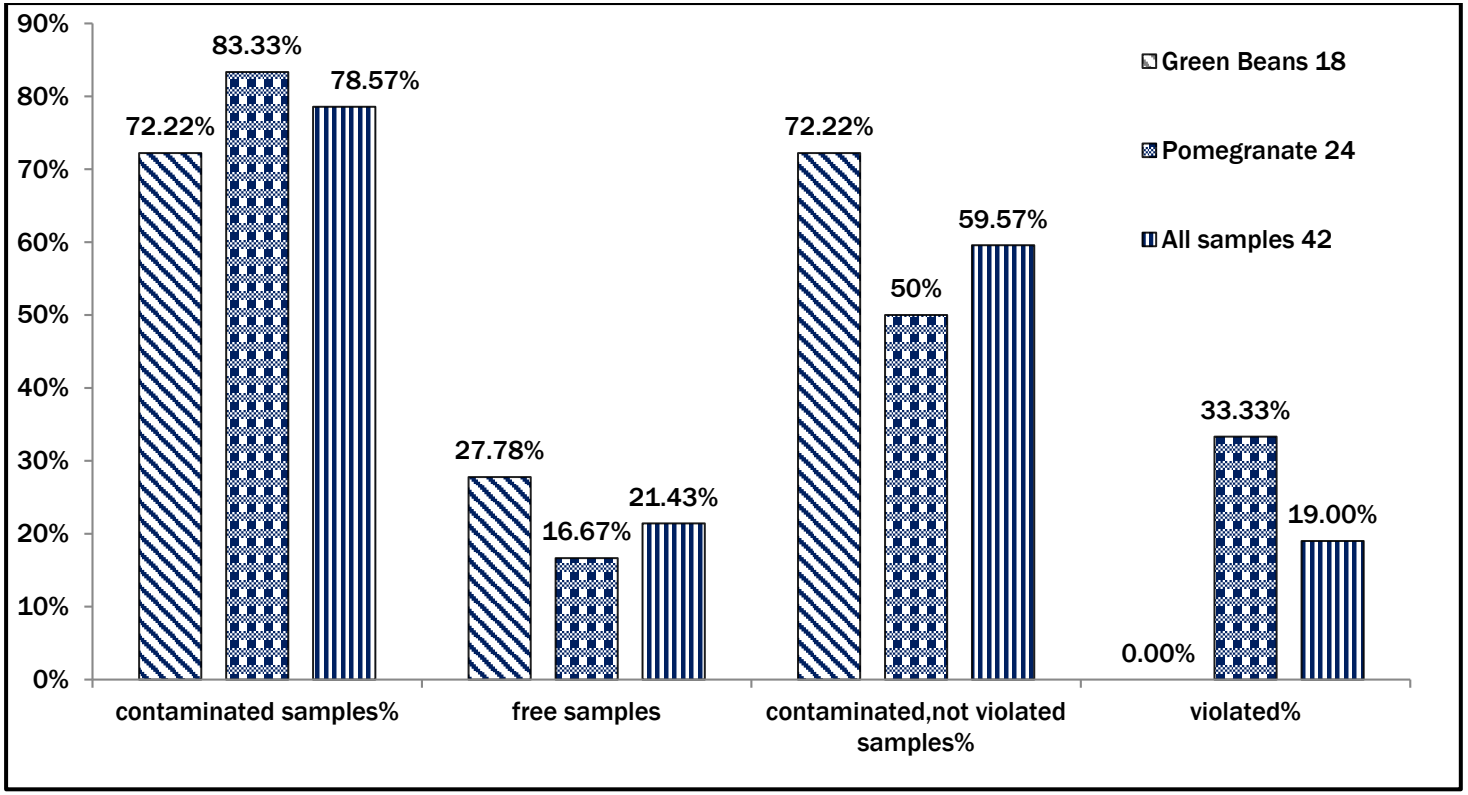

Fig.1: The contamination and the violation percentages in green beans and pomegranate samples collected from markets in six centers in Minia Governorate (EGYPT) during 2018.

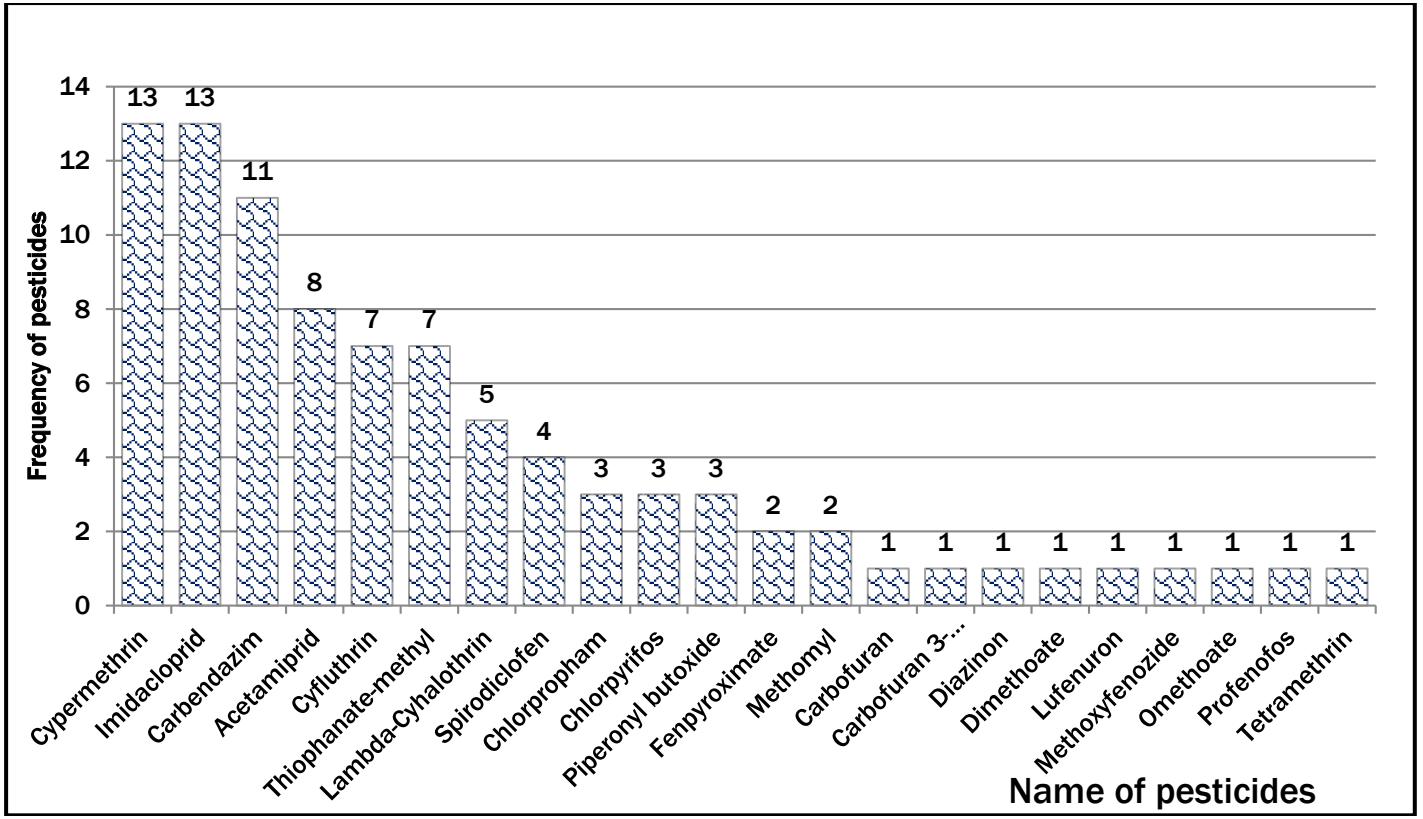

Fig. 2: Frequency numbers of residues found overall concentration ranges in green beans and pomegranate samples collected from markets in six centers in Minia Governorate (EGYPT) during 2018.

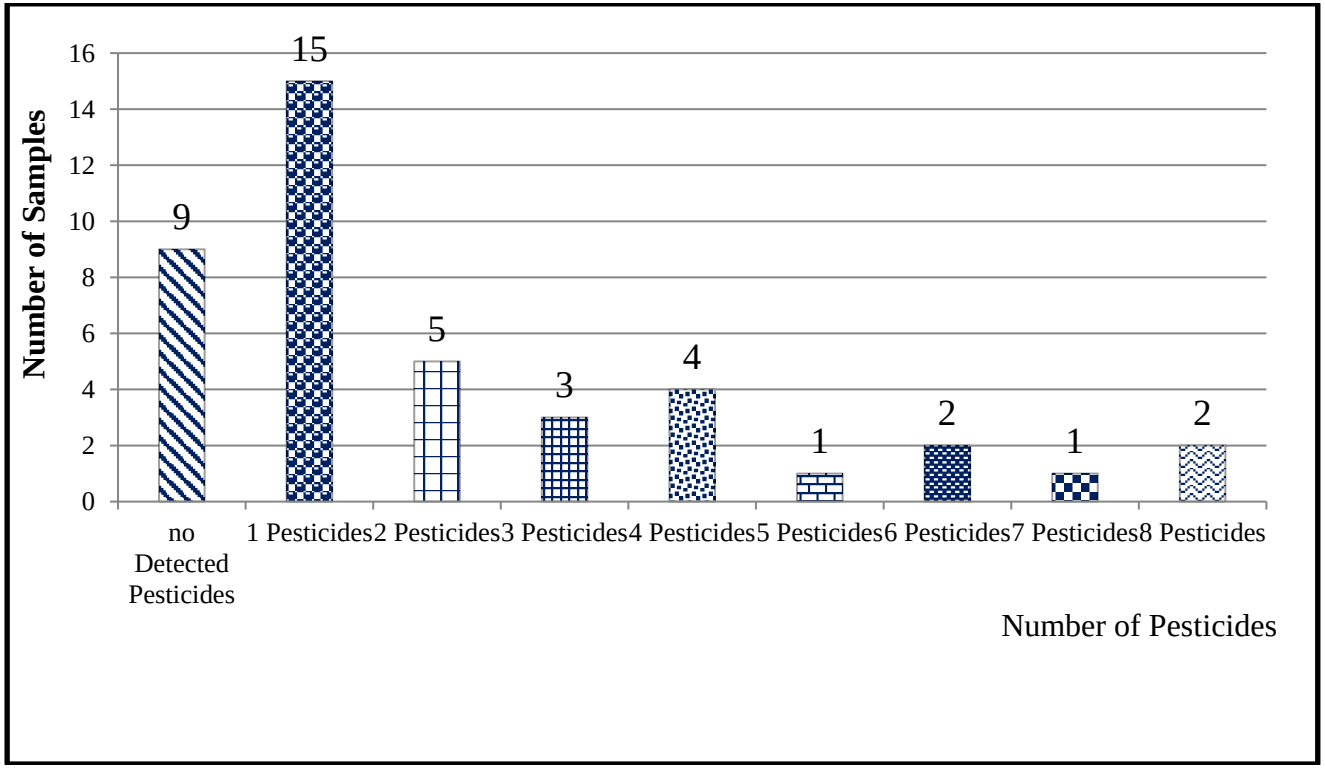

Fig. (3) Number of pesticide detected in green beans and pomegranate samples

Discussion*:*

The Agricultural Pesticides Committee (APC) is the Egyptian competent authority responsible of the registration of agricultural Pesticides, the committee issued the recommendation of registered Pesticides for each crop, (APC ,2019). However, there were a lot cases where not authorized Pesticides were found in green beans and pomegranate whereas, out of nine Pesticides found in green beans, six pesticides registered but not recommended for using on green beans including Carbendazim, Chlorpropham, Lambda-cyhalothrin ,Profenfos and Thiophanate-methyl while one pesticide was registered and recommended i.e. Imidacloprid. There were also two products not registered i.e. Tetramethrin and Piperonyl butoxide. In pomegranate samples, out of seventeen Pesticides found, five Pesticides were not registered in Egypt i.e. Carbofuran, Carbofuran-3-hydroxy, Cyfluthrin, Diazinon and Omethoate while twelve Pesticides were registered but not recommended i.e. Acetamiprid, Carbendazim, Chlorpyrifos, Cypermethrin, Dimethoate, Fenpyroximate, Imidacloprid, Lambda-cyhalothrin ,Lufenuron, Methomyl, Spirodiclofen and Thiophanate-methyl.

The pesticides found in both green beans and pomegranate were Carbendazim, Imidacloprid, Lambda cyhalothrin and Thiophanate methyl.

These previously findings indicated misuse, random applications of pesticides and Lack of awareness of the farmers about the dosage, right ways of application and the suitable interval between harvesting and pesticide treatment. The carelessness or non-availability of correct guidance concerning the pesticide application may be another reason for pesticide residues in the green beans and pomegranate samples. These contaminated samples are may be potential health risks to the consumers. The misuse or overuse of pesticides without any prior guidance and knowledge are become series problems. The obtained data from green beans showed higher percentage of contamination with pesticide residues (i.e.78.57%) when compared with those obtained previously by Dogheim, *et al.,* (1999 and 2001), GadAlla *et al.,* (2013) and Radwan, *et al.,* (2016) which found that the percentage of contamination were 41.6 ,25.5 ,23.1 and 44.12%,respectively. Table (5) there were not violation rates in our study, while in the previous studies they were 0.00, 2.48, 7.69 and 3.81%. In comparison, the rate of contamination with pesticide residues in pomegranate which obtained by Dogheim *et al.,* (2001) was 46.4%, the corresponding violative rate was 3.6% Table (5). The current study had higher contamination rate i.e.83.3% and higher violative rate for pomegranate samples i.e.33.33%.

This study provides scientific evidences of detected residues of many pesticides in green beans and pomegranate. The important findings are the observed high rate of pesticide residues detected and high incidence of pesticide residues detection

exceeding their MRL's indicating that the GAP may not be well followed.

Attention should be paid for Egyptian agriculture authorities (APC) to improve the management of pesticide use and control. In addition, well developed training programs should be initiated to improve pesticide application knowledge for farmers and monitoring of pesticide residues in vegetables and fruits should be performed on a routine basis. The results are also helpful for the risk assessment of consumers exposure to those pesticide residues.

.

Recommendations:

1- Monitoring programs for pesticide residues in all food products should be maintained in order to provide and update a data base through which future strategies for pest control and consumer protection against indiscriminate exposure to pesticides can be implemented.

2-To prioritize the development of strategies to reduce pesticides in agriculture by training farmers to use pesticides in a wise and secure manner and to encourage the use of alternatives to chemicals in the control of pests such as biological control.

3-Compliance with the rules of application of pesticides especially pre harvest interval (PHI).

Table (5): Comparing the current results with some previous monitoring pesticide residues data carried out in green bean and pomegranate samples in Egypt.

Monitoring years	Green Beans		Pomegranate		References
	Contamination%	Violation %	Contamination%	Violation %	
1995	41.6	--------			Dogheim *et al.*, (1999)
1996	25.5	2.48	46.4	3.6	Dogheim *et al.*, (2001)
2011	23.1	7.69			GadAlla *et al.*, (2013)
2015	44.21	3.81			Radwan *et al.*, (2016)
2018	72.2	--------	83.3	33.33	Current work

REFERENCES:

APC,.(2019).http:w.w.w.apc.gov.eg/en/default.aspx.

Beena, K. V. K. Madan and T. S. Kathpal,.(2006). Monitoring of Pesticide Residues in Fruits. Journal of Environmental Monitoring and Assessment, 123: 1-3, pp. 407-412.

Beena, K. and T. S. Kathpal,.(2009). Monitoring of Pesticide Residues in Vegetarian Diet," Journal of Environmental Monitoring and Assessment, 151: 1-4, pp. 19-26.

Bempah, C. K. and A. K. Donkor,.(2011).Pesticide Residues in Fruits at the Market Level in Accra Metropolis, Ghana, a Preliminary Study," Journal of Environmental Monitoring and Assessment, 175: 1-4, pp. 551-561.

CODEX,.(2010). Codex alimentarius commission Pesticide residues in food and feed. Downloaded from http: //www. codexalimentarius.net /pestres/ data/ pesticides/index.html Codex Alimentarius Commission ,1993. Joint FAO/WHO Food Standards Programme, 2, sec. 4, p. 391.

Dogheim, S. M. ; Alla, S. G. ; El-Marsafy, A. M., and Fahmy, S. M. (1999). Monitoring pesticide residues in Egyptian fruits and vegetables in 1995. JOURNAL-AOAC INTERNATIONAL, 82, 948-955.

Dogheim, S.M. ; S.A., Gad Alla and A.M. El-Marsafy,.(2001). Monitoring of Pesticide Residues in Egyptian Fruits and Vegetables during 1996. J. AOAC Int., 84, (2) 519-531.

Dogeim, S. M.; S.A.Gadalla ;E. Y. Salama ; A. M. El-Marsafy and Y. M.Nabil, .(2002). Monitoring of pesticide residues in Egyptian fruits and vegetables during 1997.Food Additive and Contaminants 19(11): 1015-1027.

European Committee for Standardization/Technical Committee 275, 2007. Food of plantorigin: prEN 15662: Determination of pesticide residues using GC-MS and/or LC-MS (/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE-QuECHERS method.European Committee for Standardization, Bussels.

Freidberg, S. (2003). Cleaning up down South: Supermarkets, Ethical Trade and African Horticulture. Social and Cultural Geography. 4(1): pp. 27-43.

GadAlla, S. A. ;Thabet, W. M. and Salama, E. Y. (2013). Monitoring and risk assessment of pesticide residues in some Egyptian vegetables. Middle East Journal of Applied Sciences, 3, 216-230.

Gambacorta, G. ; M. Faccia ; C. Lamacchia ;A. Di Luccia, and E. La Notte,.(2005). Pesticide residues in tomato grown in open field. Food Control. 16: 629 – 632.

Jiang, Y. F. and X. T. Wang ; Y. Jia,.(2009). Occurrence, Distribution and Possible Sources of Organochlorine Pesticides in Agricultural Soil of Shanghai, China," Journal of Hazardous Material, Vol. 170, No. 2-3, pp. 989- 997.

Kocourek, V. ; J. Hajsˇlova´; K. Holadova´and J. Poustka,.(1998). Stability of pesticides in plant extracts used as calibrants in the gas chromatographic analysis of residues. J Chromatograph,800: 297–304.

Krol, W. J. ; T. L. Arsenault and H. M. Pylypiw,.(2000). Reduction of Pesticide Residues on Produce

by Rinsing," Journal of Agriculture and Food Chemistry, Vol. 48, No.10, pp. 4666-4670.

Mebdoua, S. (2018). Pesticide Residues in Fruits and Vegetables. In J.-M. Mérillon & K. G. Ramawat (Eds.), Bioactive Molecules in Food (pp. 1-39). Cham: Springer International Publishing.

Radwan, O. M. M. ; Khorshed M. A. ; El-Gohary A. A. and Sorour ,M. (2016); Monitoring of Some Pesticide Residues in Consumed Green Beans in Egypt, Middle East Journal of Applied Sciences Volume : 06 | Issue : 01 | Jan.-Mar. | 2016 Pages: 70-77 ISSN 2077-4613 .

Rissato, S.R.; M.S. Galhiane ; M.V.Almeida ; M. Gerenutti and B.M.Apon,.(2007). Multi residue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. Food Chemistry, 101: 1719 – 1726.

Stachniuk, A.; Szmagara, A. ;Czeczko, R. and Fornal, E. (2017). "LC-MS/MS determination of pesticide residues in fruits and vegetables Part B Pesticides, food contaminants, and agricultural wastes." Journal of environmental science and health v.52 no.7 pp. 446-457. .

Thabet, W. M.; Shendy, A. H.and Gadalla, S. A. (2016). Chronic exposure of insecticide and fungicide as indicator of health impact in some commonly consumed leafy vegetables: Case study. *Cogent Food & Agriculture*, *2*(1), 1193926.

Zambonin, C.G.; M. Quinto; N. De Vietro and F. Palmisano,.(2004). Solid-phase microextraction – gas chromatography mass spectrometry: A fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices. Food Chemistry, 86: 269 – 274.

Zhang, Z.Y.; X.J. Liu ; X.Y. Yu ; C.Z. Zhang and X.Y. Hong,.(2007). Pesticide residues in spring gabbage grown in open field. Food Control, 18: 723-73.

رصد بعض بقايا المبيدات في الفاصوليا الخضراء والرمان بمحافظة المنيا (مصر)

إسراء عبدالله محمد خلف [1]، جمال ابو المكارم رزق [1] ، منير محمد محمود ألماظ [2]

[1] قسم وقاية النبات، كليه الزراعة، جامعه المنيا، جمهورية مصر العربية
[2] المعمل المركزي للمبيدات مركز البحوث الزراعية

تم إجراء الدراسة الحالية لإتمام تحقيق برنامج رصد بسيط ،حيث بهدف تقصي متبقيات المبيدات في عينات من الفاصوليا الخضراء والرمان المجمعة من محافظه المنيا (مصر) خلال أكتوبر لسنه 2018، حيث تم جمع 42 عينه من الفاصوليا الخضراء والرمان من 6أسواق محليه في مراكز محافظة المنيا (بني مزار– مطاي – سمالوط – المنيا – أبو قرقاص – ملوي) وتم استخلاص العينات بطريقة(QuEChERS) وتحليلها باستخدام جهاز الكروماتوجراف السائل المتصل بمطياف الكتلة المتتابع (LC–MS/MS) وجهاز الكروماتوجراف الغاز المتصل بمطياف الكتلة المتتابع(GC–MS/MS) لمتبقيات المبيدات. أظهرت النتائج أن 21.43% من العينات الكلية كانت خاليه تماما من متبقيات المبيدات في حين تم رصد 59.57% من العينات بها متبقيات المبيدات من غير أي تعدي للحدود القصوى وكان 19% من العينات تحتوي على متبقيات مبيدات متعديه للحدود القصوى. وقد سجلت الفاصوليا الخضراء أعلي نسبه من التلوث بمتبقيات المبيدات ولكن دون تعدي للحدود القصوى للمبيدات (72.22%) يليها الرمان (50%)، بينما سجل الرمان أعلي نسبه من التعدي للحدود القصوى (33.33%). أظهرت النتائج أن المبيدات الأكثر تواجدا في عينات الفاصوليا الخضراء والرمان هي سايبرمثرين(13) يليها إيميداكلوبريد(13) ثم كاربيندازيم(11) وأسيتامبريد(8) وسايفلوثرين(7) وثيوفانات ميثيل(7) ولمباداثيهالوثرين(5) وسبيرودايكلوفين(4) وكلوربروفام(3) وكلوربيريفوس(3) و فينبيروكسيميت(2) وأخيرا ميثوميل(2) بالإضافه إلي تواجد المادة المنشطة للمبيدات ببرونيل بيوتوكسيد ثلاث مرات . وكانت المبيدات الأقل تواجدا والتي تواجدت مره واحدة فقط هي كاربوفيوران و كاربوفيوران 3–هيدروكسي و ديازينون و ديميثوات ولوفينورون و ميثوكسيفينوزيد وأوميثوات و بروفينوفوس وأخيرا تيترامثرين . ولقد أظهرت النتائج أيضا أن المبيدات المتعدية للحدود القصوى في الرمان سبعه مبيدات وهي (أسيتامبريد – سيفلوثرين–كلوربيريفوس–ديميثوات–أوميثوات–ميثوميل وثيوفانات–ميثيل)، كما أظهرت النتائج أن العينات الأكثر تلوثا كانت العينات المأخوذة من سوقي المنيا ومطاي يليها سوق أبوقرقاص ثم سوق بني مزار يليه سوق ملوي ثم سوق سمالوط وأخيرا مزرعة بملوي. كما لم يتم تسجيل اي قيم للتلوث في مزرعة بأبو قرقاص. وأيضا أظهرت النتائج أن 15 عينه من العينات المجمعة للفاصوليا الخضراء والرمان احتوت على متبقيات لمبيد واحد وعينتين احتوت علي متبقيات ل8 مبيدات مختلفة.