

Multi-residue pesticides in conventional and organic orange

Abstract: a monitoring project for pesticide residues in orange samples in Al-Tamer vegetables market, Riyadh was conducted. A total of 144 samples of organic and non-organic orange were collected according to the Codex Alimentarius recommendations. Samples were extracted with acetonitrile and subjected for clean-up using Florisil column. Clean extracts were analyzed using GC-MS against 86 pesticides of concern. Method performance parameters are reported. Organic orange samples of both countries contained non-detectable amounts of the tested pesticides. For the non- organic produce samples it contained varying amounts of pesticides depending on the season, country and month. Mostly, insecticides were found mostly in the samples then fungicides. Violating pesticides were also the anti-insect ones. Methomyl was the violating compound in South African orange and ethion in Egyptian one. Fungicides were below the corresponding MRL. Total amount of residues were the maximum in December 2010 (5.16 mg/kg) followed by November 2010 (4.27 mg/kg) of which ethion was the major constituent, this may be due to insect control practices. The highest level of residues appeared in the November 2011 with 1.68 mg/kg residues of methomyl followed by august 2011 with 1.3m mg/kg consist of methomyl and chlorpyrifos-methyl.

Key words: GC-MS, pesticide residues monitoring, multi-residues analysis, orange

1-Introduction

Increased demands are being placed on farmers to produce food more efficiently and safely. Food safety is a major public concern worldwide. The major pathway of human exposure to pesticides is food ingestion followed by inhalation and dermal contact (Fries, 1995). Freshly consumed Vegetables and fruits, are or high concern, as they do not receive any cooking treatments, increasing the problem of pesticide residues (Solecki et al., 2005). A risk assessment is necessary to ascertain the health effects due to intake of pesticide residues

in food. Pesticides are used during production and post-harvest treatment of agricultural commodities to guarantee higher productivity of crops and better quality of food (FAO/WHO, 2013). Due to the poor pesticide handling practices and use of more toxic pesticides, inadequate management and regulation of these chemicals in developing countries (Chen et al., 2011), pesticide contamination levels are higher in the developing countries than the developed ones (Bhanti and Taneja, 2007). Control programs for pesticide residues in poor countries are limited either for lack of resources or proper legislations are not set. Many farmers do not observe the set Pre-Harvest Interval (PHI) because of their high demand for farm produce and low perception of the toxic effects of pesticide residues in food (Amoha, et al., 2006). Increased use of chemical pesticides has resulted in occurrence of residues in food commodities (Darko and Akoto, 2008) and contamination of the environment (Calvert et al., 2001). Pesticide residues have been linked with a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption (Berrada et al., 2010).

Pesticide residue monitoring is the only tool to control the quantity of pesticides on food. For the past few decades regulatory authorities in many countries have been setting up monitoring systems for the agricultural products and the environment. The surveillance focuses on the proper use of pesticides in terms of authorization and registration (application rates and pre-harvested intervals), and on compliance with maximum residue limits (MRLs). Pesticide residue monitoring is also recognized as a significant aspect of initiatives to reduce potential hazards to human health (Blasco et al., 2005, Dogheim et al., 2002 and Fernández et al., 2001).

Fruits and vegetables have been given a lot of attention in monitoring programs since most of them are eaten raw, it is expected that they contain higher pesticide residue levels compared to other food groups of plant origin. According to the Pesticide Residues

Committee in the UK, consumers are encouraged to eat at least five portions of fruits and vegetables daily. Therefore, assessing the risk of pesticide residues in these commodities intended for human consumption is necessary.

This study aimed to monitor pesticide residues in terms of type and quantity in orange fruits imported from Egypt and South Africa as two major producing and supplying countries of Saudi Market. The study targeted Navel orange produced organically or by conventional farming.

2- Materials and Methods

2-1- Reagents and equipment

All pesticides standard were obtained from (Riedel de Haen and Supelco). 1mg/ml stock solution of each was prepared by dissolving 20mg of the pure analytical standard in acetone. A single composite standard solution was prepared by diluting with acetone according to limit of detection (LOD). All standard solutions were stored in glass-Stoppard flasks at 4°C. Mixed compound calibration solutions were prepared in acetone and they were used as spiking solution. Solvents (residue analysis grade) used were acetone, acetonitrile, petroleum ether and other reagents such as sodium chloride and anhydrous sodium sulphate, florisil 60-100 mesh for residue analysis were also from purchased from (Fluka). The florisil and anhydrous sodium sulphate was activated at 100 °C overnight and stored in 500 ml glass flask with glass stoppers and stored in oven at 100 °C. The equipment used were a high-speed blender with a stainless steel jar (Waring, USA), a separation funnel shaker (GFL, Germany), a rotavapor, R 215 with cooler circulator chiler B-740 (Buchii, Switzerland), Buchner funnel and chromatographic tubes with Teflon stopcocks and coarse fritted glass (Agilent, USA) and syringes (Hamilton Bonadus AG, Switzerland). All glassware were rinsed thoroughly using soap and deionization water, then washed with acetone and dried in oven (100-130 °C) over night.

2-2- Sampling

Samples were collected from Al-Tamer Market, Riyadh, Saudi Arabia. Egyptian Orange (Navel) samples were collected during the active production season months from November 2009 to February 2010 and from November 2010 to February 2011. In case of South African orange (Navel), samples were collected during the active production season from July to October 2010 and from July to October 2011. Samples were collected from non-organic orange (conventional agriculture product) and organically produced orange. Codex Alimentarius procedures were followed in sampling and transporting. Samples were collected monthly and were kept in polyethylene bags in fridge at 4 °C until analyzed.

2-3- Sample preparation

The chopped orange subsamples (100g) were placed in a stainless steel jar 1L and extracted with 200ml of acetonitrile and 10g celite, the mixture was vigorously homogenized at high speed for 2 min. and the mixture was filtrated using Buchner funnel fitted with shark-skin filter paper into 500 ml suction flask. Organic extract was transferred to 1L separator funnel and 100ml of (PE) was added, the mixture was vigorously shaken for 2 min and then 100ml saturated solution of NaCl and 600 ml water were added. The mixture was vigorously mixed and the separator funnel was allowed to be held at horizontal position for few minutes. The aqueous layer was discarded and the solvent layer was washed twice with 100 ml portions of distilled water and the washed layer was transferred into 100 ml beaker and 15g of anhydrous sodium sulphate was added and filtered through Whatman filter paper. Finally the extract was concentrated to 5ml in rotary evaporator and transferred directly to florisil column for clean-up. Column was prepared according to the AOAC method (1995, chap. 10). The column was contained about 12 cm activated florisil topped with 1cm anhydrous sodium sulphate, column was washed by 40ml (PE) and then sample was added and allowed to pass through the column. The walls of the tube were rinsed additional small portions of petroleum

ether and elute at 5ml/min with 200ml 6% eluting solvent (Diethyl ether in (PE)), and then 200ml 15% and finally 200ml 50% eluting solvent (Diethyl ether in (PE)) at 5ml/min. column effluent was collected, solvent was evaporated and residue was transferred to injection vials then subjected to determination step.

2-4- Instrumentation

Gas chromatograph-mass spectrometer (Aglient model 6890N) coupled with (model 5975B) quadruple mass spectrometer with a GC column HP-5MS 5% phenyl - 95% methyl siloxane, 30m x 0.25mm id x 0.25 μ m film thickness was used (plate 3). GC operating conditions: splitless injection, injector temperature 250 °C, helium carrier gas (99.9999 purity) at flow rate 0.9 ml/min with column head pressure 7.4 psi, oven temperature from 70 °C (2 min hold), than raised to 130 °C at the rate (25 °C/min) afterwards raised to 220 °C at (2 °C/min) and then raised to 280 °C at (10 °C/min) and eventually (4.6 min hold). The sample (1 μ L) was injected in splitless modes. The MS system was routinely set in selective ion monitoring (SIM) mode and each compound was quantified based on peak area using one target ion (quantifier) and one or two qualifying ion. Mass spectrometer parameter was set as follows: electron impact ionization mode with 70 eV electron energy, scan mass range 100-400 at 0.62 sec/cycle. Ion source temperature 230 °C, MS quad temperature 150 °C, EM voltage 1450 and solvent delay time is 4 min.

2-5- Recovery

Orange samples, free of tested pesticides, were used for the fortification experiments. 100 g homogenized sample was spiked prior to determination procedure by addition of a mixture of standard pesticides solution 0.1 μ g/ml of each compound. Spiked samples were left to stand for 30 min. to allow pesticide to absorb into the sample. Samples were extracted according to the method described above. The limit of detection (LOD) and recoveries data for tested pesticides are tabulated in table 1.

2-6- Determination of pesticides residues

An appropriate aliquot (2µl) of samples was injected in Gas Chromatography Mass spectrum under the mentioned conditions and then pesticides residues had been identified by comparison of retention time values with reference standard. Confirmation of results was performed using selected ion monitoring (SIM) mode, one is the quantifier and the qualifying ion. Table 3 shows the retention time and characteristic masses (m/z) for each pesticide.

3- Results and discussion

The method used for analysis has been published before (Selim, et al., 2011) and it was checked for performance appropriateness before analysis. Linearity, recovery LOQ and reproducibility parameters were tested.

3-1- Pesticide residues in conventional (non-organic) orange samples

3-1-1- Egyptian Product

Data in table 2 summarize average levels of pesticide residues in the collected representative samples of orange imported from Egypt during the active production season from November 2009 to February 2011 for 2 successive seasons. Out of 86 tested pesticides only nine compounds were found i.e. Chlorpyrifos-methyl, Pirimiphos- methyl, Dimethoate, Ethion, Propamocarb, Permethrin, Fenvalerate, Dicofol, Vinclozolin. Detected compounds belong to different pesticide chemical groups, organophosphorus, pyrethroids, organochlorine and dicarboximides. Mostly they possess insecticidal/acaricidal activity only Vinclozolin in a fungicide, this may be attributed to the fact that insecticides are used much more than fungicides in open field, similar results are obtained by Sameeh et al., 2009. Figure 1 is showing the number of violating samples (exceeding MRL), which was 5 times for Ethion and 4 times for dicofol. Chlorpyrifos-methyl, propamocarb and vinclozolin were lower than MRL in all samples during the two tested seasons. Samples of February 2009 scored the

highest record of detection times of pesticides as 7 pesticides were detected three of them were higher than the MRLs. Total amount of residues were the maximum in December 2010 (5.16 mg/kg) followed by November 2010 (4.27 mg/kg) of which ethion was the major constituent, this may be due to insect control practices as shown in figure 2.

3-1-2- South African Product

Residues found in South African orange are tabulated in table 3. Samples were collected during the active production season from July 2010 to November 2011. The found pesticides are mostly insecticides as in the Egyptian Product data above. The detected pesticides were the insecticides; Chlorpyrifos-methyl, primiphos-methyl, dimethoate, methomy and the fungicides; propamocarb and benodanil. The most violating pesticide was methomyl with 5 records of exceeding limits followed by dimethoate (3 times) and chlorpyrifos-methyl (3 times). Both fungicides were below the limits during both tested seasons as shown in figure 3. The highest level of residues appeared in the November 2011 with 1.68 mg/kg residues of methomyl followed by august 2011 with 1.3m mg/kg consist of methomyl and chlorpyrifos-methyl (figure 4). July 2011 and October 2011 samples have 3 detection records. All samples contained pesticides residues at least once.

3-2- Pesticide residues in organically produced orange

Fortunately, all organic samples collected from the Egyptian or the South African products were free of tested pesticides to the limit of quantification of the method reported above (table 1). These results are very much encouraging for consuming organic orange from both countries, with taking in consideration the price issue.

In conclusion, a total of 144 samples of organic and non-organic orange samples collected for 2 seasons from Saudi market imported from Egypt and South Africa. Organic orange samples of both countries contained non-detectable amounts of the tested pesticides.

For the non- organic produce samples it contained varying amounts of pesticides depending on the season, country and month. Mostly, insecticides were found mostly in the samples then fungicides. Violating pesticides were also the anti-insect ones. Methomyl was the violating compound in South African orange and ethion in Egyptian one. Fungicides were below the corresponding MRL. The number of detected pesticides in Egyptian orange (9) was higher than South African product (6). Total residues in Egyptian orange were higher than the product of South Africa.

References

1. Fries, G.F., 1995. A review of the significance of animal food products as potential pathways of human exposures to dioxins. *J. Animal Sci.* 73 (6), 1639–1650.
2. Solecki, R., Davies, L., Dellarco, V., Dewhurst, I., Raaij, M. V., and Tritscher, A. (2005). Guidance on setting of acute reference dose (ARfD) for pesticides. *Food and Chemical Toxicology*, 43, 1569-1593.
3. WHO/GEMS/FOODS (WHO/Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program). (2013). Consumption cluster diets. <http://www.who.int/foodsafety/chem/gems/en/>
4. Chen, C., Q. Yongzhong, Q. Chen, C. Tao, C. Li, Y. Li (2011). Evaluation of pesticide residues in fruits and vegetables from Xiamen, China. *Food Control*, 22, 1114-1120.
5. Bhanti, M., and Taneja, A. (2007). Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. *Chemosphere*, 69, 63-68.
6. Amoah, P., Drechsel, P., Abaidoo, R. C., and Ntow, W. J. (2006). Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Archives of Environmental Contamination and Toxicology*, 50, 1-6.

7. Darko, G., and Akoto, O. (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi, Ghana. *Food and Chemical Toxicology*, 46, 3703-3706.
8. Calvert, G. M., Sanderson, W. T., Barnett, M., Blondell, J. M., and Melher, L. (2001). Surveillance of pesticide-related illness and injury in humans. In R. Krieger, J. Doull, D. Ecobichon, E. Hodgson, L. Reiter, & J. Ross (Eds.), *Handbook of pesticide toxicology* (2nd ed.). (pp. 603-641) New York, NY: Academic Press.
9. Berrada, H., Fernández, M., Ruiz, M. J., Moltó, J. C., Mañes, J., and Font, G. (2010). Surveillance of pesticide residues in fruits from Valencia during twenty months (2004/05). *Food Control*, 21, 36-44.
10. Blasco, C., Font, G., & Picó, Y. (2006). Evaluation of 10 pesticide residues in oranges and tangerines from Valencia (Spain). *Food Control*, 17, 841-846.
11. Dogheim, S. M., El-Marsafy, A. M., Salama, E. Y., Gadalla, S. A., and Nabil, Y. M. (2002). Monitoring of pesticide residues in Egyptian fruits and vegetables during 1997. *Food Additives and Contaminants*, 19, 1015-1027.
12. Fernández, M., Pico, Y., & Manes, J. (2001). Pesticide residues in oranges from Valencia (Spain). *Food Additives and Contaminants*, 18, 615-624.
13. Selim, M.T., M.H. EL-Saeid and I.M. Al-Dossari (2011). Multi-residues analysis of pesticides using gas chromatography mass spectrometry: I- leafy vegetables. *Research Journal of Environmental Sciences*, 5 (3), 248-285.
14. Sameeh, A. Mansour, M. H. Belal, A.K. Abou-Arab, M. F. Gad (2009). Monitoring of pesticides and heavy metals in cucumber fruits produced from different farming systems. *Chemosphere*, 75, 601–609.

Table 1. Parameters of retention time, LOD, recovery percent and target and qualifier ions m/z in SIM mode

Compounds	Retention time min	LOQ	Recovery %		Target ion m/z	Qualifier ions m/z	
			Clay soil	Sandy soil		Q ₁	Q ₂
Dichlorvos	7.211	0.02	59	64	109	185	79
Propamocarb	9.849	0.04	78	98	58	71	129
Mevinphos	10.828	0.09	63	49	127	192	109
Chloroneb	13.015	0.01	79	77	191	193	206
Methomyl	14.837	0.01	44	53	105	88	57
Propachlor	16.350	0.02	65	96	120	77	176
Propoxur	16.440	0.03	77	91	110	152	81
Ethoprophos	17.183	0.05	72	64	157	97	139
Bendiocarb	18.808	0.01	63	100	151	126	166
Sulfotep	19.350	0.01	50	100	322	202	97
Alfa-BHC	19.449	0.02	78	95	183	181	219
hexachlorobenzen	19.949	0.05	66	75	284	249	142
Dichloran	20.412	0.03	32	65	176	206	124
Dimethoate	20.694	0.02	46	43	87	93	125
Simazine	21.240	0.01	63	98	201	186	173.2
Carbofuran	21.517	0.01	50	72	164	149	123
Lindan	21.953	0.01	58	100	219	181	111
Fonofos	22.837	0.06	56	79	109	137	246
Delta-BHC	23.934	0.05	91	47	181	219	111
Diazinon	24.181	0.03	66	36	179	137	152
Iprobenfos	25.472	0.04	47	93	91	204	122
Pirimicarb	26.194	0.05	71	57	166	72	238
Dichlorfenthion	26.869	0.01	39	63	279	223	162
Phosphamidon I	27.074	0.03	80	100	127	72	264
Phosphamidon II	27.105	0.05	59	66	127	72	264
Chlorpyrifos-Me	27.530	0.03	86	71	286	125	288
Vinclozolin	27.643	0.02	36	88	212	285	187
Carbaryl	27.846	0.03	59	100	144	115	116
Alachlor	28.292	0.04	71	63	160	188	146
Ronnal	28.738	0.02	60	97	285	287	125
Metalaxyl	28.894	0.05	63	102	206	146	192.2
Fenitrothion	30.004	0.07	45	58	277	125	109
Linuron	30.118	0.04	41	72	61	187	124
Aldrin	30.417	0.02	57	103	66	263	91
Thiobencarb	30.794	0.08	76	49	100	72	125
Malathion	31.310	0.03	81	85	127	173	99
Fenthion	31.679	0.03	58	100	278	125	109
Pirimiphos-ethyl	34.279	0.05	49	93	318	333	304
Captan	34.791	0.06	76	98	79	151	114
Chlorofenvenphos	35.549	0.05	78	95	267	323	269
Chlordan-trans	35.990	0.05	64	100	373	375	237
Alfa-endosulfan	36.919	0.03	98	107	239	237	195
Nanchlor-trans	37.168	0.02	49	100	409	100	237
Chlordane-cis	37.311	0.01	77	98	375	373	377
Disulfoton sulfon	37.670	0.01	53	97	213	153	97
Dieldrin	39.172	0.02	96	100	79	265	81
P,P-DDE	39.688	0.03	91	90	246	318	248
O,P-DDD	40.321	0.04	64	67	235	237	165
Endrin	40.920	0.01	44	95	263	265	281
Beta-endosulfan	41.820	0.03	97	63	207	239	195
Chlorobenzilate	42.683	0.01	49	84	251	139	253
P,P-DDD	43.234	0.04	91	49	235	237	165
Benodanil	43.773	0.03	94	73	231	323	203
Ethion	44.131	0.03	47	89	231	97	153
Carbophenothion	45.558	0.04	71	37	157	121	125
Resmethrin I	48.965	0.03	65	71	123	171	143
Resmethrin II	49.532	0.04	49	43	123	171	143
Hexabromobenzen	49.772	0.02	52	59	551.6	554	549.6
Phosmet	50.300	0.07	118	71	160	161	77
EPN	50.726	0.02	49	100	157	169	185
Dicofol	50.955	0.05	97	96	139	111	251
Fenoxycarb	51.040	0.01	88	93	255	186	116
Tetramethrin II	51.322	0.04	73	68	164	123	81
Tetradefon	52.029	0.03	77	29	159	111	229
Mirex	52.460	0.05	50	89	272	274	270

Furathiocarb	52.620	0.03	46	55	163	57	164
Amitraz	53.373	0.04	62	69	132	121	147
Lamda-cyhalothrin	53.770	0.04	73	83	181	197	208
Azenophos-ethyl	53.882	0.04	21	77	132	160	77
allethrin I	54.515	0.02	60	67	123	181	81
allethrin II	54.523	0.02	49	102	123	181	81
allethrin III	54.590	0.02	65	91	123	181	81
Permethrin I	54.891	0.05	89	100	183	163	165
Permethrin II	55.111	0.04	99	93	183	163	165
Comaphos	55.165	0.04	101	99	263	226	109
Cyfluthrin III	55.855	0.04	77	89	163	165	226
Cyfluthrin I	55.997	0.03	94	100	163	165	226
Cyfluthrin IV	56.103	0.03	100	102	163	165	226
Cyfluthrin II	56.162	0.03	97	93	163	165	226
Cypermethrin II	56.284	0.03	87	100	163	165	181
Cypermethrin IV	56.424	0.02	84	100	163	165	181
Cypermethrin I	56.522	0.02	105	97	163	165	181
Cypermethrin III	56.575	0.05	59	99	163	165	181
Fenvalerate I	57.454	0.03	66	36	125	167	281
Fenvalerate II	57.716	0.04	47	93	125	167	281
Deltamethrin	58.440	0.03	87	100	253	181	

Table 2. Pesticides residues detected (mg/kg) in orange imported from Egypt during (2010- 2011)

Detected pesticides	November		December		January		February		MRL	V
	(2009)	(2010)	(2009)	(2010)	(2010)	(2011)	(2010)	(2011)		
Chlorpyrifos-methyl	nd	nd	0.667	0.017	0.302	0.036	0.38	0.002	0.50	0
Pirimiphos- methyl	nd	0.644	0.179	nd	1.146*	nd	0.185	0.012	1.00	1
Dimethoate	1.06*	nd	nd	0.173*	nd	nd	0.076*	nd	0.02	3
Ethion	nd	3.581*	0.542*	3.666*	nd	0.176*	nd	0.035*	0.01	5
Propamocarb	nd	nd	nd	nd	0.004	nd	0.056	nd	0.1	0
Permethrin	Nd	0.045	0.014	nd	nd	nd	0.742*	0.742*	0.05	2
Fenvalerate	0.0586*	nd	nd	0.0066	0.065*	0.176*	nd	nd	0.02	3
Dicofol	0.173*	nd	nd	1.306*	nd	nd	2.146*	1.435*	0.02	4
Vinclozolin	Nd	nd	0.0033	nd	0.0233	nd	0.0133	nd	0.05	0
No. of detections	3	3	5	5	5	3	7	5		
Total residues detected (ppm)	1.29	4.27	1.40	5.16	1.53	0.388	3.59	2.226		

Each figure is an average of 3 replicates

nd: not detected

*violating sample

V: number of violating samples

Table 3. Pesticides residues detected (mg/kg) in non-organic orange imported from South Africa (2010- 2011)

Detected pesticides	July		August		September		October		November		MRL	V
	(2010)	(2011)	(2010)	(2011)	(2010)	(2011)	(2010)	(2011)	(2010)	(2011)		
Chlorpyrifos-methyl	0.026	0.185*	nd	0.666*	nd	nd	0.055*	nd	nd	nd	0.05	3
Pirimiphos- methyl	0.0123	nd	nd	nd	0.066	nd	nd	nd	0.379	nd	1.00	0
Dimethoate	nd	0.38*	0.076*	nd	nd	nd	nd	0.326*	nd	nd	0.02	3
methomyl	nd	nd	1.180*	0.665*	nd	0.433*	nd	0.073*	nd	1.681*	0.02	5
Propamocarb	nd	nd	nd	nd	nd	nd	0.007	nd	nd	nd	0.1	0
benodanil	nd	0.038	nd	nd	nd	0.007	nd	0.25	nd	nd	0.50	0
No. of detections	2	3	2	2	1	2	2	3	1	1		
Total residues detected (ppm)	0.037	0.418	1.256	1.332	0.066	0.440	0.062	0.649	0.379	1.681		

Each figure is an average of 3 replicates

nd: not detected

*violating sample
V: number of violating samples

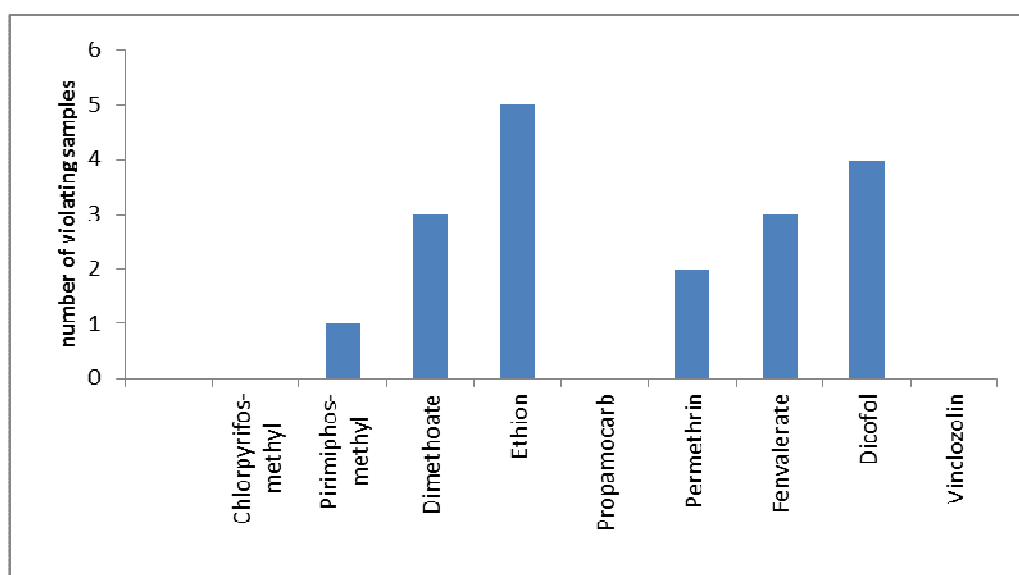


Figure 1. Number of violating Egyptian orange samples of each detected pesticides in the whole study samples

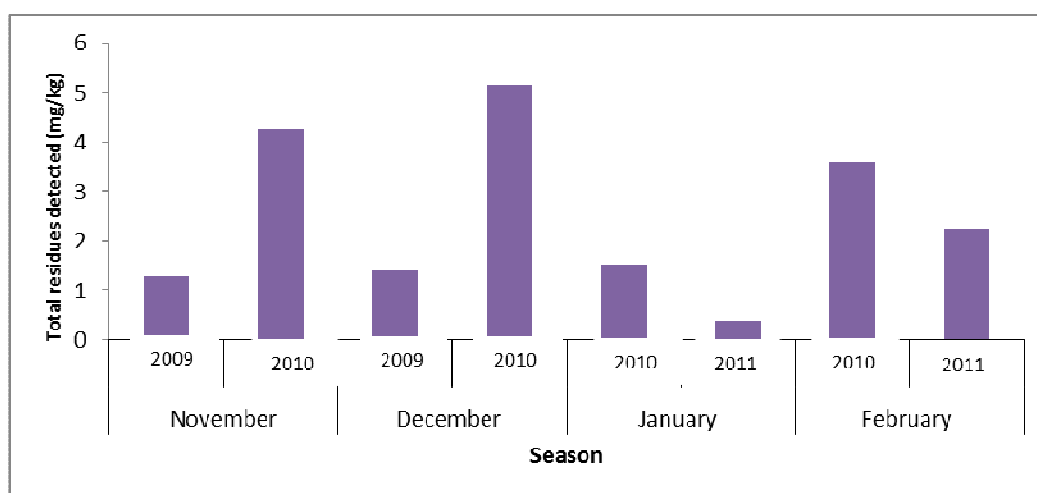


Figure 2. Comparison of total pesticide residues found in each month in both seasons of the study in Egyptian orange

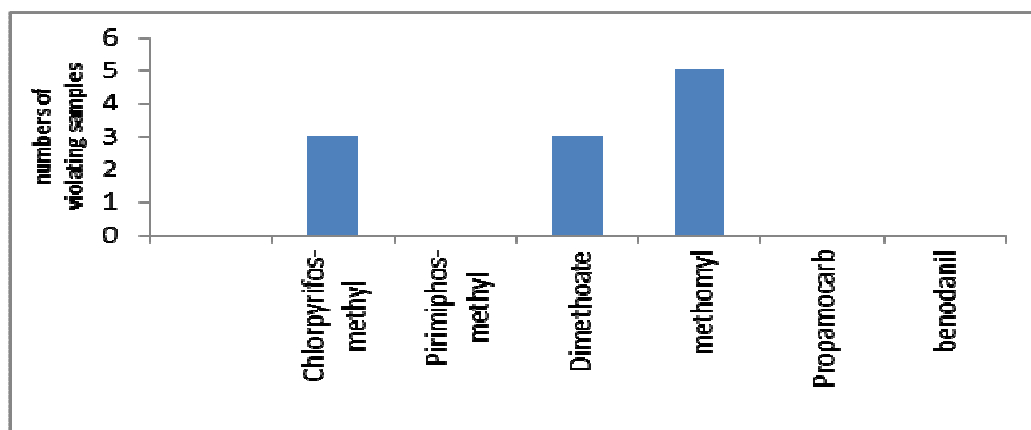


Figure 3. Number of violating South Africa orange samples of each detected pesticides in the whole study samples

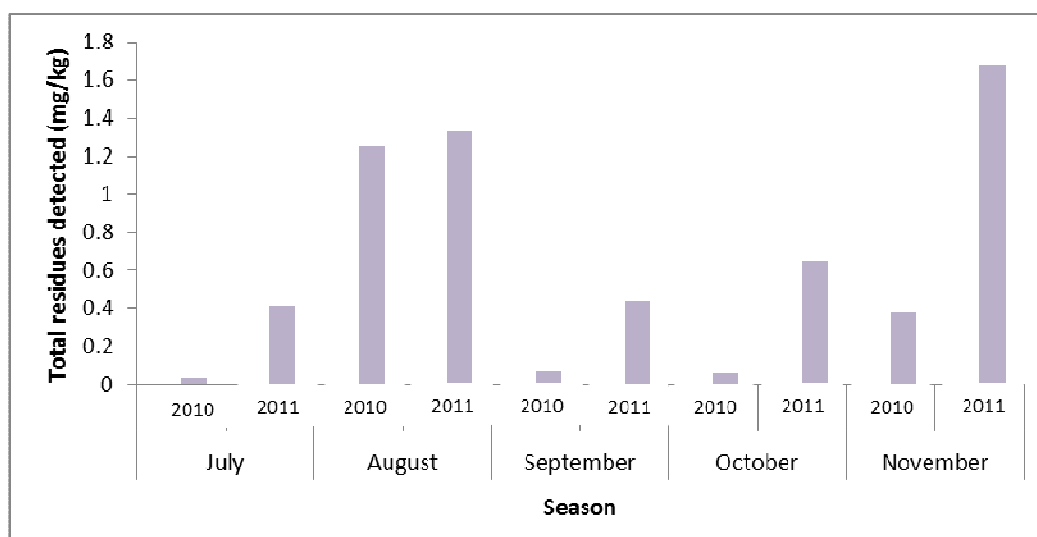


Figure 4. Comparison of total pesticide residues found in each month in both seasons of the study in South Africa orange