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Determination of 107 Pesticide Residues in Citrus Fruits by Gas Chromatography/Mass Spectrometry

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This study was carried out to investigate the organochlorine (OC), organophosphorus (OP) and organochlorine and organophosphorus (OC and OP) pesticide residues in citrus fruit samples (mandarin, orange and lemon) collected from the Aegean and Mediterranean regions of Turkey. Sample preparation and extraction were performed using Luke extraction method. The concentrations of pesticides were determined by gas chromatography with selective detectors: electron capture detector, nitrogen phosphorus detector and mass selective detector and confirmed with mass-spectrometry. At least one type of pesticide residue was found in 105 samples out of 210 (50 %). In 5 samples (2.4 %), the residue levels were found above the maximum residue levels of Turkish food codex and European union standards.

Key Words: Quinalphos, Citrus fruit, GC/MS, Imazalil, Pesticide residue.

INTRODUCTION

Citrus fruits include sour orange, orange, mandarin, grapefruit, bergamot and lemon. In Turkey, they are grown especially in Mediterranean and Aegean regions and also partially in east black sea region. While 70 % of total citrus fruits are produced in Cukurova region, Izmir has priority in the production of Aegean region and provides 5 % of total production[1].

The chemical and biological materials that are harmful for human, environment, health and/or products and prevent, repel, destroy and/or mitigate the organisms expressed as 'pest' are defined as pesticide[2]. Pesticides are chemical materials used for prevention, destroying and control of unwanted insects during the growing, storage, transportation, distribution of agricultural products or during the process of foods and agricultural products[3].

In the production of citrus fruits, certain prevention practices are needed against to several types of diseases and insects like red and yellow louses have a shell, floury louses, leaf louses, leaf fleas, lemon flower moth, carob moth, white fly, cochineal insect have bag, star cochineal insect, Mediterranean fruit fly, rust insect, citrus bud acarus[1].

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The usage of pesticides and determination of the residue levels in the environment are important subjects because of the toxic, mutagenic, carcinogenic, teratogenic characteristics of these chemicals. Other than the accidental cases, human can be damaged directly by the pesticides at the steps of transportation, storage, usage and consumption of foods which include pesticide residue[4]. Therefore, the determination of pesticide residue levels in agricultural products is a matter of great concern in recent years. Pesticide residue surveillance program and EUREPGAP (Good Agriculture Practice Standards in European Retail Sector) are good examples that indicate sight of view of Eurepean union for this concern. The maximum residue levels (MRLs) for pesticides in foodstuffs were defined in certain legislations such as the EU directives[5] or Turkish food codex (TFC)[6] to ensure food safety for consumers.

GC with specific detectors such as electron capture detector (ECD), nitrogen phosphorus detector (NPD) and mass selective detector (MSD) has been the most widely used techniques for many years[7-12].

The aim of this study was to determine the occurrence of organochlorine (OC), organophosphorus (OP) and OC and OP pesticide residues in 210 citrus fruit samples taken from Aegean and Mediterranean regions in Turkey. GC/ECD, GC/NPD and GC/MS were used for the determination of OC, OP and OC and OP pesticides, respectively.

EXPERIMENTAL

Organochlorine pesticide analysis were performed on Agilent 6890N (Palo alto, CA, USA) GC with ECD. The GC was fitted with HP-5 capillary column with a length of 30 m, inner diameter of 0.32 mm and film thickness of 25 μm. Injection, oven and detector temperatures were 230, 220 and 250 ºC, respectively. Helium was used as a carrier gas with a flow rate of 1.3 mL min^{-1} and the process time was 60 min.

Agilent 6890N GC/NPD (Palo Alto, CA, USA) with 30 m × 0.25 mm i.d. PAS 1701 column was used for OP pesticide. The following oven temperature program was employed: initial temperature was 200 ºC, increased at 10 ºC min^{-1} to 250 ºC, held for 20 min; increased at 10 ºC min^{-1} to 280 ºC, held for 10 min. Detector temperature was 240 ºC. Helium was used as a carrier gas with a flow rate of 1.5 mL min^{-1}. The process time was 58 min.

Organochlorine and organophosphorus pesticides (OCP and OPP) analysis were performed on Agilent 6890N 597 inert GC/MS (Palo alto, CA, USA). The GC was fitted with HP-5 MS silica capillary column (5 % phenyl methyl siloxane as non-polar stationary phase, 30 m × 250 μm film thickness) from Agilent (Palo alto, CA, USA). Helium was used as a carrier gas with a flow rate of 1.3 mL min^{-1}. It was flown with a rate of 50 mL min^{-1} for 2 min in valve and 20 mL min^{-1} for 2 min in gas conservative. The following oven temperature program was implemented: initial temperature of 70 ºC held for 2 min; increased at 25 ºC min^{-1} to 150 ºC held for 0 min then increased at 3 ºC min^{-1} to 200 ºC held for 0 min; then another ramp 8 ºC min^{-1} to 280 ºC, held for 10 min.

The injector temperature was 250 ºC. The process time was 61.87 min. The residue concentrations of samples were determined from the area of peaks at chromatography using calibration curve. In the sample preparation step, Heidolp Laborata 4000 (Germany) evaporator was used.

Stock standard solutions with different concentrations were prepared from 107 types of pesticide agent material standards which were obtained from Ehrenstorfer GmbH (Augsburg, Germany). The purities of the standard pesticides varied between 99 % and 99.5 %. Sigma-Aldrich (Germany) acetone of 99.8 % purity, AnalaR (England) sodium sulphate of 99.9 % purity, Carlo Erba (Germany) petroleum ether of 99.8 % purity, Riedel-de Haen (Germany) dichloromethane of 99.8 % purity, J.T. Baker deionized water of 99.9 % purity, Wako (Osaka, Japan) sodium chloride and Supelco (Bellefonte, PA, USA) flourosil column were used for extraction.

Sampling: 210 citrus fruit samples (70 mandarins, 70 oranges and 70 lemons) collected from Izmir (Seferihisar, Karaburun, Menderes), Mugla (Ortaca, Koycegiz, Fethiye), Antalya (Kumluca, Finike, Demre, Kemer) and Mersin (Erdemli, Silifke) were used as research materials. Two kg of each sample were supplied from local markets, producers and markets at different dates following to the harvesting period randomly in triplicate. The pesticide-free samples were used as controlling group and analyzed previously.

Preparation of samples: Samples were homogenized by mechanical miller in 2 kg batches and weighted for extraction. Luke extraction method was used for organochlorine pesticides and organophosphorus pesticides analysis because there was no oil in the samples. 200 mL acetone was added to 50 g sample taken from homogenized sample and mix was filtered. 80 mL filtered liquid was taken and filtered again with sodium sulphate and then put into separating funnel. It was shaked after adding 100 mL petroleum ether, 100 mL dichloromethane and sodium chloride which was saturated with water. After phase separation, lower part was transferred to another separation funnel. Upper part was filtered with sodium sulphate and transferred to balloon. Lower part was shaked with dichloromethane two times and then it was transferred to balloon too. Collected extract was evaporated at < 45 ºC and passed from conditioned flourosil column. Extract was evaporated again at < 45 ºC and solved with 5 mL acetone and then transferred to vials. Vials were injected to GC according to pesticide group[13-15].

Evaluation: The retention times of pesticides were detected with prepared stock standard solutions of different concentration and then calibration curve was drawn. Residue concentration of analyze samples was determined with using this calibration curve. The recoveries and the detection limits are presented in Table-1.

RESULTS AND DISCUSSION

In this study, at least one pesticide residue was found in 50 % of the total citrus fruit samples (105 of 210 samples). None of the 105 citrus fruit samples (50 % of the total samples) examined containing pesticide residue. One or more pesticide

TABLE-1
RECOVERY AND DETECTION LIMITS (DLs) OF
107 PESTICIDES IN CITRUS FRUIT SAMPLES (n = 3)

Pesticide's name	Recovery	DLs (mg kg^{-1})	Pesticide's name	Recovery	DLs (mg kg^{-1})
2,4'-DDE	97	0.005	Flamprop-methyl	78	0.030
2,4'-DDT	71	0.005	Formothion	87	0.010
4,4'-DDD	106, 72	0.070	Heptachlor	93, 9	0.050
4,4'-DDE	100	0.020	Heptachlor endoepoxide (isomer A)	68, 29	0.029
4,4'-DDT	60, 25	0.045	Heptachlor exoepoxide (isomer B)	74	0.001
Alachlor	95	0.010	Hexachlorobenzene	62	0.010
Aldrin	51, 080	0.040	Imazalil	71	0.020
α-HCH	104, 240	0.008	Iodofenphos	78	0.010
α-endosulfan	92, 270	0.007	Iprodione	52	0.020
Azobenzene	80	0.010	Lindane (G-HCH)	147, 835	0.004
Azinphos-methyl	68	0.020	Linuron	83, 33	0.050
β-HCH	86, 925	0.072	Malaoxan	87	0.050
β-endolsulfan	95, 850	0.040	Malathion	97, 53	0.031
Bromophos-ethyl	59	0.020	Metalaxyl	83	0.050
Bromophos-methyl	78	0.020	Methamidophos	128	0.010
Bromopropylate	100	0.010	Methidathion	100	0.020
Bupirimate	91, 66	0.030	Methoxychlor	90, 08	0.088
Buprofezin	61	0.060	Methyl-parathion	82	0.050
Captan	95	0.010	Metribuzin	80	0.010
trans-Chlordane	80	0.010	Monocrotophos	50	0.010
Chlorfenapyr	84	0.010	Myclobutanil	50	0.020
Chlorfenson	81, 33	0.050	Nuarimol	93, 66	0.010
Chlorfenvinphos	95, 22	0.010	Oxy-chlordane	102	0.010
Chlorothalonil	80	0.020	Penconazole	99	0.025
Chlorpyrifos	101, 025	0.014	Pentachloroaniline	93	0.010
Chlorpyrifos-methyl	86, 38	0.007	Procymidone	87, 63	0.009
Chlorpropham	83	0.060	Propargite	100	0.010
cis-Chlordane	77	0.010	Propyzamide	58	0.050
Coumaphos	76	0.030	Pyrazophos	107	0.050
δ-HCH	96, 605	0.003	Pyrimethanil	78	0.020
Demeton-*s*-methyl	84	0.010	Omethoate	125	0.050
Diazinon	71, 51	0.097	Oxadixyl	71	0.100
Dichlorvos	99, 126	0.007	Quintozene	87	0.020
Dicofol	97	0.010	Paraoxon-ethyl	100, 33	0.100
Dicrotophos	41	0.010	Pendimethalin	66	0.060
Dieldrin	86, 170	0.099	Phosalone	118	0.025
Dimefox	55	0.010	Phosphamidon	96, 99	0.032
Disulfoton-sulfoxide	70	0.050	Pirimiphos-ethyl	72, 97	0.082
Ditalimfos	81	0.010	Primiphos-methyl	109	0.010
Endosulfan-sulfate	161, 455	0.119	Quinalphos	92	0.010
Endrin	90, 505	0.044	Simazine	69	0.050

Endrin aldehyde.	105, 460	0.008	Sulprofos	96	0.010
Endrin ketone	127, 855	0.027	Tebuconazole	71	0.020
Ethion	112	0.010	Tecnazene	82	0.015
Ethofumesate	87, 66	0.100	Tetrasul	93, 33	0.050
Ethoprophos	45	0.500	Tolclofos-methyl	84, 43	0.050
Ethyl-parathion	50	0.010	Triadimefon	87, 89	0.050
Fenamiphos	58	0.050	Triazophos	61	0.010
Fenarimol	58	0.010	Trichlorfon	74	0.010
Fenchlorphos	62	0.010	Trifluralin	89, 38	0.025
Fenitrothion	97	0.010	Tetraconazole	44, 66	0.050
Fenson	86, 33	0.050	Triadimenol	76, 33	0.050
Fenthion	67	0.010	Vinclozolin	104	0.025

residue was detected in 38 mandarin (54.3 %), 31 orange (44.3 %) and 36 lemon samples (51.4 %) at or under TFC[7] and EU MRLs. The residue exceeding TFC[7] and EU MRLs was only found in 5 lemon samples (2.4 %) of the total samples; 7.1 % of the lemon samples (Fig. 1).

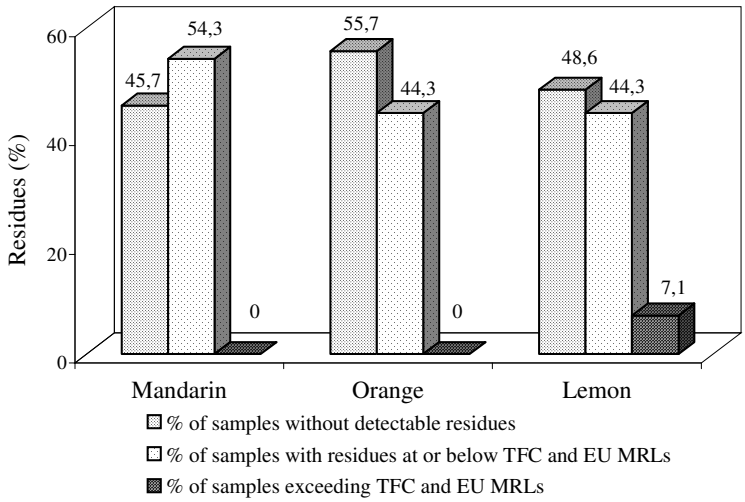

Fig. 1. Changes of detected pesticides according to Turkish Food Codex (TFC) and European Union Maximum residue levels (EU MRLs)

The mean levels of five detected pesticides in citrus fruit samples are presented in Table-2. Imazalil residues were detected in 6 mandarin, 20 lemon and 21 orange samples with mean levels of 0.162, 0.083 and 0.162 mg kg^{-1}, respectively (Fig. 2). Quinalphos residues were detected in two mandarins with a mean level of 0.0245 mg kg^{-1}, 5 oranges with a mean level of 0.023 mg kg^{-1} and 26 lemons with a mean level of 0.028 mg kg^{-1}, respectively. On the other hand, in 5 lemon samples, quinalphos residue exceeding TFC[7] and EU MRLs were detected from 0.055 to 0.078 mg kg^{-1}. Also chlorpyriphos residues were detected in 8 mandarin and 11 orange

TABLE-2
MEAN PESTICIDE RESIDUES IN CITRUS FRUIT SAMPLES (mg kg^{-1} fresh wt)

Samples	Imazalil	Quinalphos	Chlorpyriphos	Bromopropylate	Malathion
Mandarin (n = 70) (38)[a]	0.162 (5.0/5.0)[b] (0.024-0.494)[c] (6)[d]	0.0245 (0.05/0.05) (0.024-0.025) (2)	0.016 (2.0/2.0) (0.014-0.019) (8)	0.020 (2.0/3.0) (0.011-0.028) (6)	BDL[e]
Orange (n = 70) (31)	0.162 (5.0/5.0) (0.031-0.298) (21)	0.023 (0.05/0.05) (0.014-0.025) (5)	0.020 (0.3/3.0) (0.014-0.028) (11)	0.020 (2.0/3.0) (0.013-0,028) (4)	0.032 (2.0/2.0) (0.031-0.033) (2)
Lemon (n = 70) (36)	0.083 (5.0/5.0) (0.025-0.226) (20)	0.028 (0.05/0.05) (0.010-0.078) (26)	0.018 (0.2/2.0) (0.014-0.021) (11)	BDL	BDL

[a]No. of samples one or more residues, [b]limits in TFC / EU MRL (mg kg^{-1}), [c]detected range, [d]No. of detected samples, [e]BDL below detection limit.

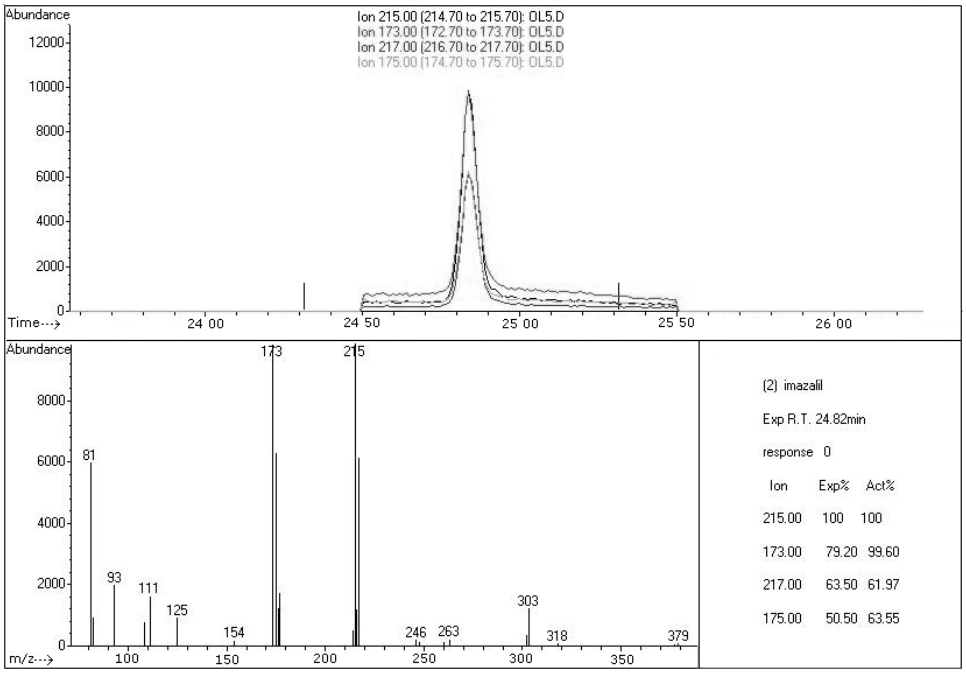

Fig. 2. Representative chromatogram of imazalil

and lemon samples with mean levels of 0.016, 0.020 and 0.018 mg kg^{-1}. Furthermore, bromopropylate residues were detected in 6 mandarin and 4 orange samples with a mean level of 0.020 mg kg^{-1}. For malathion, a mean residual concentration of 0.032 mg kg^{-1} was found in only 2 orange samples.

The pesticide residue level was found under the TFC[7] and EU MRLs in the 54.3 % of mandarin, 44.3 % of orange and lemon samples. The pesticide level was found above the TFC and EU MRL in the 7.1 % of lemon samples. There have been many studies reporting pesticide residue levels in fruits and vegetables. In accordance to the EC commissions directives as part of the pesticide residue trace programmes, the residue above the national and international MRL value was detected in the member countries in 3 % of total 6021 fruits and vegetables in 1997[16], 3.3 % of 5174 samples in 1998[17], 4.3 % of 4707 samples in 1999[18], 4.5 % of 3696 samples in 2000[19], 4.3 % of 9868 samples in 2001[20], 5.5 % of 10046 samples in 2002[21] and 3 % of 8579 samples in 2003[22]. Similarly, Tatli[3] found the pesticide residue in 31.81 percentages of total 128 fresh fruits, vegetables and dried food samples. Also, in 3 samples, the residue levels were detected above the TFC and EU MRL. In addition, Taga and Daglioglu[23] analyzed total 110 citrus samples in terms of OC, OP, OC and OP and synthetic pyretroit pesticide groups and they detected the residue in the 82.73 % of these samples and also they found the residue above the TFC and EU MRL in the 4.54 % of this portion. The levels and distribution obtained from this study are in agreement with these previous survey and routine monitoring results.

Conclusion

In conclusion, a fast, easy, cheap and efficient multi-residue method based on Luke extraction method and GC analysis has been applied for citrus fruit samples. The method had been successfully applied to routine analysis of 210 citrus fruit samples. Aegean and Mediterranean regions provide a big part of the fruit production especially citrus fruits production in Turkey. In addition, these regions almost provide all of the exported citrus fruit. The precautions must be taken about the levels of pesticides in these products which are grown in these regions; so the residue level must be under the national and international MRLs. For this purpose, EUREPGAP applications which are practiced obligatorily for 10 years at EU member countries must be carried out in Turkey for all producers.

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