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Organophosphorus Insecticide Residues in Honey Produced in Turkey

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Abstract In this study, a number of 15 organophoshorus (OP) insecticides were investigated in 275 honey samples in 33 different cities of Turkey, using gas chromatography electron capture detector. The limit of determination values was detected between 0.25 and 9.55 ng g^{-1}. The correlation coefficients obtained from calibration curves of the OP standards were found to be between 0.992 and 0.999. No insecticide residue was detected in the samples analyzed. This result is highly significant because of its impacts on public health and food safety.

Keywords Honey · Gas chromatography · Organophosophorus insecticides · Residue

Beekeeping involves the collection and management of honeybees (*Apis mellifera* L.) for specific objectives. The history of modern beekeeping goes back 20–30 years. Honeybees produce beeswax, royal jelly, venom, pollen and propolis, and assist pollination in plants (MARA 2002; Zeybek 1991).

Some substances used for pest management break easily down and become inactive, while others (organochlorine (OC) compound, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), metals, and some fungicides) are hazardous because of their slow degradation in nature (Kaya et al. 2002b). Drug residues occur in honey in two ways; one via the treatment of bee diseases by the use of drugs, the other through the applications of insecticides in agriculture (Kaya et al. 2002a). The maximum acceptable residue limit (mrl) of total pesticides in honey for Turkish Codex is 0.01 mg kg^{-1} (Turkish Food Codex 2005). The aim of this study was to find a suitable laboratory method for determination of the residues of organophosphorus (OP) insecticides in honey produced in Turkey.

This article was summarized from Yavuz Kursad Das' PhD thesis.

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Materials and Methods

Two hundred and seventy-five honey samples from 33 different locations in a variety of cities of Turkey were analyzed in 2004. They are displayed in Fig. 1.

The OP insecticides investigated hereby were dichlorvos, trichlorphon, dimethoate, diazinon, parathion-methyl, chlorpyrifos-methyl, malathion, chlorpyrifos, parathion-ethyl, chlorfenvinphos, methidathion, ethion, tetradifon, phosalone and coumaphos. The insecticide standards were purchased from Dr.Ehrenstorfer (Germany). Florisil, n-hexane, dichloromethane, cyclohexane, isooctane, and methanol were purchased from J.T.Baker (USA). Acetone was purchased from Lab-Scan (Ireland). Anhydrous sodium sulfate was purchased from Kimetsan (Turkey). Agilent gas chromatography (6890 N) with electron capture detector and 30 m × 0.32 mm capillary column coated with a 0.25 μm thick film of phenyl-methyl silicon were used for the analysis of honey samples. A rotary evaporator (Heidolph Laborta 4002) was used to evaporate

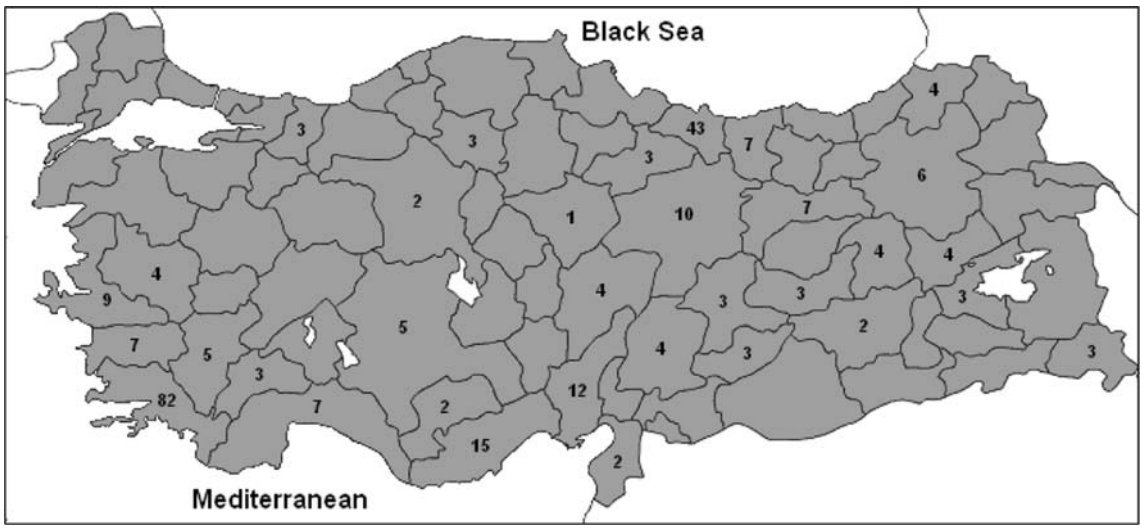

Fig. 1 Honey samples which were collected from 33 different cities in Turkey

solutions. An incubator (Dedeoglu Sti.) was used for the activation of florisil.

Four standard concentrations (50, 100, 500, 1,000 ng g^{-1}) were injected to the GC-ECD for every insecticide. Correlation coefficients were calculated automatically. Signal to noise (S/N) values were quantified for every standards. LODs and LOQs were calculated from S/N values by using the correction factors three and ten, respectively. For quantity of recovery percentages, a total of 10 g honey sample that was free of insecticide residues was measured in a beaker. It was heated at 35°C in a water bath. It was cooled to room temperature. Three spiked amounts of standard mixtures of 15 OP insecticides, namely, 100, 500 and 1,000 µg kg^{-1} were spiked into 10 g of blank honey sample. Recovery percentages were detected automatically.

Before analysis, florisil was activated by heating at 130°C for 2 days. A total of 10 g florisil was cooled to room temperature (25°C) and tightly filled in column. A total of 2 g anhydrous sodium sulfate was added to florisil. For conditioning, 50 mL *n*-hexane/dichloromethane (1:1, v/v) was passed through the column, and finally 1 g honey was dissolved in 2 mL methanol. After conditioning the column, the sample mixture was spilled into the florisil column that was conditioned previously. After the sample mixture was passed, insecticide residues were eluted with 30 ml n-hexane/dichloromethane (1:1, v/v) from the column. The eluate was collected in a balloon and evaporated at 30°C temperature and decreased pressure (300 mbar). After the eluate was drained, it was dissolved in 1 mL methanol (Jimenez et al. 1998).

The oven temperature of Gas Chromatography with electron capture detector (GC-ECD) programs were as follows: Initial temperature 60°C, held for 2 min, a 10°C min^{-1} ramp to 160°C, a 2°C min^{-1} ramp to 250°C, held for 10 min. The flow rate of carrier gas (N_2) was 1.5 ml min^{-1}. One microliter splitless injection was carried out at 240°C. The purge valve was 1 min. The ECD temperature was 300°C (Pelosi et al. 2002).

Results and Discussion

Fifteen OP insecticide standards were injected into GC-ECD. Their retention times are shown in Table 1, correlation coefficients obtained from calibration curves of the OP insecticides are displayed in Table 2, and the GC-ECD chromatogram is depicted in Fig. 2. The LOD and LOQ values of insecticide standards are shown in Table 3, and the recovery percentages and relative standard deviations (RSD) of insecticide standards are displayed in Table 4 as follows.

In this study, recovery values of some insecticides have been determined to be more than 100%. This is because of

Table 1 Retention times of OP insecticides

Insecticide	Retention time (min)	Insecticide	Retention time (min)
Dichlorvos	10.421	Parathion-ethyl	26.587
Trichlorphon	10.425	Chlorfenvinphos	29.524
Dimethoate	13.942	Methidathion	30.468
Diazinon	20.758	Ethion	37.103
Parathion-methyl	23.281	Tetradifon	45.420
Chlorpyrifos-methyl	23.292	Phosalone	46.317
Malathion	26.013	Coumaphos	52.507
Chlorpyrifos	26.557		

Table 2 Correlation coefficients obtained from calibration curves of the OP insecticide standards

Insecticide	Correlation coefficient	Insecticide	Correlation coefficient
Dichlorvos	0.992	Parathion-ethyl	0.998
Trichlorphon	0.998	Chlorfenvinphos	0.992
Dimethoate	0.993	Methidathion	0.999
Diazinon	0.999	Ethion	0.995
Parathion-methyl	0.997	Tetradifon	0.999
Chlorpyrifos-methyl	0.997	Phosalone	0.997
Malathion	0.994	Coumaphos	0.994
Chlorpyrifos	0.998		

the fact that some substances in the samples have the same retention time as do the insecticides. Due to this effect, called matrix effect, peaks of the substances increase their heights and areas by collaborating with the peaks of insecticides. This problem, seen frequently in complex samples such as honey, has also been reported previously (Jimenez et al. 1998).

In this study, no residues of 15 OP insecticides were found in 275 honey samples from 33 different cities in Turkey.

Drug residues in food are regulated by Codex Alimentarius. There have been no residue limits of drugs for honey, as determined by Codex Alimentarius so far (CODEX STAN 2001). Because of that there have been some contradictions occurring in international trade because there are different residue limits accepted in different countries (Blasco et al. 2003). European Commission regulated the use of drugs and varied substances residues in live animals and their products by releasing 96/23/EC directive on 29 April 1996. Turkey must comply with the regulations of this directive because it aims to join the European Union (EU). The residue groups required for honey according to this directive are Group B1 (Antibacterial substances, including sulphonamides and quinolones), Group B2c (synthetic pyrethroids and carbamates), Group B3a (OC compounds, including PCBs) and Group B3b (OP insecticides) (EC 1996).

A number of 97 honey samples were investigated for carbaryl and cypermethrin, and no residues were found in Turkey in 2002. Also, 130 samples were analyzed in the

Table 3 LOD and LOQ values of OP insecticides

Insecticide	LOD (ng g^{-1})	LOQ (ng g^{-1})	Insecticide	LOD (ng g^{-1})	LOQ (ng g^{-1})
Dichlorvos	3.60	12.10	Parathion-ethyl	1.20	4.00
Trichlorphon	9.35	31.05	Chlorfenvinphos	0.70	2.35
Dimethoate	9.55	31.80	Methidathion	2.55	8.45
Diazinon	1.80	6.10	Ethion	0.45	1.45
Parathion-methyl	1.15	3.80	Tetradifon	0.35	1.20
Chlorpyrifos-methyl	0.35	1.20	Phosalone	4.10	13.70
Malathion	7.10	23.70	Coumaphos	1.60	5.25
Chlorpyrifos	0.25	0.75			

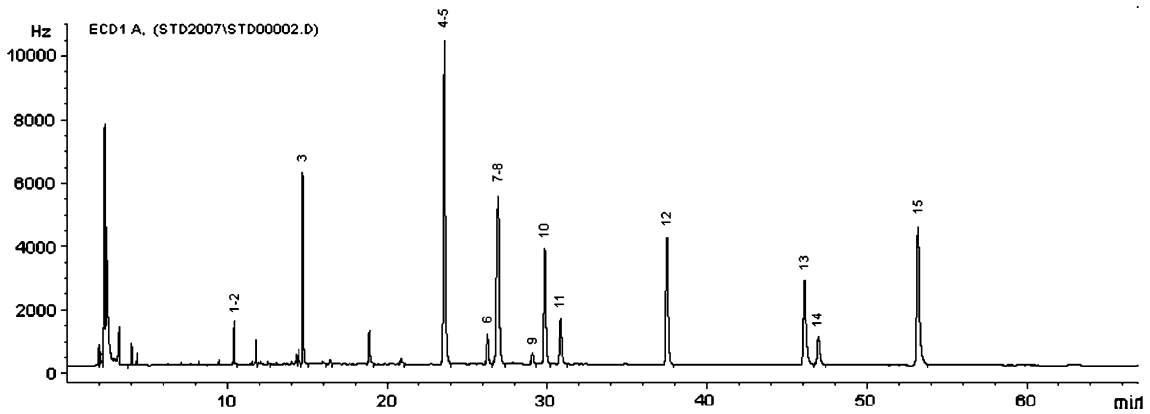

Fig. 2 The GC-ECD chromatogram of insecticides (1: Dichlorvos, 2: Trichlorphon, 3: Dimethoate, 4: Diazinon, 5: Parathion-methyl, 6: Chlorpyrifos-methyl, 7: Malathion, 8: Chlorpyrifos, 9: Parathion-ethyl, 10: Chlorfenvinphos, 11: Methidathion, 12: Ethion, 13: Tetradifon, 14: Phosalone and 15: Coumaphos)

Table 4 Recovery percentage and relative standard deviations (RSD) of insecticides

Insecticide	Recovery (%)			Mean	RSD
	1,000 ng g^{-1}	500 ng g^{-1}	100 ng g^{-1}		
Dichlorvos	146	158	292	199 (100)[a]	40.72
Trichlorphon	44	50	87	60	38.8
Dimethoate	1,170	1,126	1,262	1,186 (100)[a]	5.85
Diazinon	84	87	98	90	8.18
Parathion-methyl	19	24	47	30	49.76
Chlorpyrifos-methyl	153	123	118	131 (100)[a]	14.44
Malathion	223	253	373	283 (100)[a]	28.04
Chlorpyrifos	185	107	102	132 (100)[a]	35.25
Parathion-ethyl	68	87	73	76	12.94
Chlorfenvinphos	384	300	280	321 (100)[a]	17.19
Methidathion	38	43	51	44	14.88
Ethion	124	129	110	121 (100)[a]	8.13
Tetradifon	84	60	87	77	19.20
Phosalone	433	642	265	447 (100)[a]	42.25
Coumaphos	151	221	105	159 (100)[a]	36.73

[a] These recovery percentages are higher than 100% because of matrix effect of honey

same year for endosulpan-beta, parathion-methyl, malathion, diazinon, dichlorvos, dimethoate, trichlorphon, and coumaphos, yet no residues were detected (MARA 2003). Another 191 honey samples were searched for endosulphan, flumethrin, cypermethrin, fluvalinate, carbaryl, carbofuran, methiocarb, and methomyl in 2003, and no residue was found. Furthermore, another 148 samples were analyzed for trichlorphon, diazinon, parathion-methyl, dichlorvos, dimetoat, coumaphos and malathion, and no residues were revealed (MARA 2004). In Turkey, Selcukoglu (1999) found 1.34–33.48 μg g^{-1} amitraz residues in 25 out of 135 honey samples in 1999. No residue of fluvalinate was determined in that research.

In Spain, Garcia et al. (1996) investigated 221 honey samples during a period of between 1988 and 1991. They found 0.42 (0.033–1.82) μg g^{-1} amitraz in 19 samples, 6 (1–53) μg g^{-1} coumaphos in 32 samples, and 2 (1–15) μg g^{-1} fluvalinate in 39 samples. Fernandez Muino et al. (1997) also investigated 101 honey samples for insecticide residues. They found 5–60 ng g^{-1} bromopropilate in 16 samples, and 10–40 ng g^{-1} (100 ng g^{-1} in one of them) fluvalinate in 11 samples. They found no residues of amitraz and coumaphos (11) in all the samples. Blasco et al. (2003) analyzed 50 honey samples from Spain and Portugal, determining 0.03–0.28 μg g^{-1} α-HCH (hexachlorocyclohexane) in 14 samples, 0.01–0.27 μg g^{-1} HCB (hexachlorobenzene) in 16 samples, 0.08–3.49 μg g^{-1} β-HCH in 13 samples, 0.05–4.31 μg g^{-1} γ-HCH in 25 samples, 0.033–0.658 μg g^{-1} DDT (dichlorodiphenyltrichloroethane) in 10 samples, 0.05–0.23 μg g^{-1} heptenophos in eight samples, 0.02–0.645 μg g^{-1} carbofuran in five samples, 0.02 and 0.071 μg g^{-1} primicarb in two samples, 0.025–0.068 μg g^{-1} methidathion in four samples, 0.01 μg g^{-1} parathion-methyl in one sample, 0.003–0.027 μg g^{-1} methiocarb in seven samples and 0.016 μg g^{-1} carbaryl in one sample.

In a study performed in Belgium, 0.004 μg g^{-1} fluvalinate residue was found in one out of 215 honey samples. Also, in a study performed in Switzerland, an average of 9.8 μg g^{-1} fluvalinate residue was found in 28 out of 29 propolis samples (MAF 2001). In France, Martel and Zeggane (2002) analyzed 320 honey samples. They did not find any residue of amitraz and bromopropylate. Fluvalinate residue of 0.01–0.026 μg g^{-1} was found in three samples, and coumaphos residue of 0.11–0.26 μg g^{-1} was found in other three samples. In Jordan, Al Rifai and Akeel (1997) analyzed 26 honey samples. They did not determine any insecticide residue in two samples. They found β-HCH, α-HCH and lindane residues of 0.01–0.026 μg g^{-1} in 86% of the positive samples. They also determined OP insecticide residues in 86% of the positive samples. In Greece, Tsigouri et al. (2000) analyzed 66 beeswax samples. They determined 0.44–30.1 μg g^{-1} fluvalinate residues in all samples. In Germany, 1,000 honey samples were analyzed for insecticide residues in a study started in 1988. Bromopropilate residues were found between 2 and 10 ng g^{-1}. In the same study, coumaphos residues were found between 2 and 15 ng g^{-1} for 28% of the samples and fluvalinate residues were found between 2 and 7 ng g^{-1} for 1% of the samples (Wallner 1999). In Australia, 246 honey samples were analyzed in 1998. No residues of OC, OP and synthetic pyrethroid insecticides were detected (AFFA 1999). In Cuba, Pineiro (2003) investigated 111 honey samples in 1999 and 67 honey samples in 2000. He did not

find any residue of OC and OP insecticides and flumethrin. He found that only one sample showed positive results for OP and 12 samples for OC insecticides out of 70 samples in 2001. No residue of flumethrin was detected in the same samples. In Slovenia, Maver and Poklukar (2003) analyzed 12 honey samples in 2000. They found 0.025 μg g^{-1}, as maximum, coumaphos residue in five samples. They analyzed 19 honey samples in 2002. Coumaphos residue of 0.22 μg g^{-1}, as maximum, was found in five samples.

The results obtained in this study are similar to the results of the investigation performed in Australia in 1998 (Agriculture et al. 1999). No pesticide residues were found in 246 honey samples in that study. The findings of this study are also consistent to those of Martel and Zeggane (2002) who found that three samples showed positive results for fluvalinate residue and three for coumaphos residue. However, the results of this study differ from the studies of Blasco et al. (2003) and Al Rifai and Akeel (1997).

In this study, 275 honey samples from 33 different cities of Turkey were analyzed for 15 OP insecticides by GC-ECD. No residues of these compounds were detected in the samples. The results obtained hereby are a good indication for food safety, human health and beekeeping in Turkey. Studies in this regard are significant as it helps to determine residues in animal-originated foods, and should be performed in future too. Both animal and plant-originated foods must be investigated in terms of drug and chemical compound residues to ensure human health and food safety by annual programs. The results of this study increase the scope of export of honey from Turkey to both EU and non-EU countries.

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