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Research Article

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Investigation of pesticide ruins by LC-MS/MS and GC-MS chromatography in sweet fennel seed (*Foeniculum vulgare* Mill.)

Nilda Ersoy[1] **Volkan Okatan**[2,*] **Deniz Hazar**[3] **Ibrahim Baktir**[4]

[1]Akdeniz University, Technical Sciences, Antalya, Turkey
[2]Uşak University, Sivaslı Vocational High School, Uşak, Turkey
[3] Akdeniz University, Department of Horticulture, Antalya, Turkey
[4] Akdeniz University, Faculty of Agricultural Sciences and Technologies, Cyprus

[*]Corresponding Author: okatan.volkan@gmail.com

Abstract

The aim of present study was to investigate the effects of the pesticide residues on the seeds of sweet fennel, Foeniculum vulgare Mill. cv. Dulce, grown by good agricultural practices. The sweet fennel was grown in Tefenni town of Burdur provience in South Western Part of Turkey. The residue analyses were done by GC-MS/MS and LC-MS/MS chromatographies. Total 186 active substances in used pesticides during the cultivation period were analyzed by LC-MS/MS chromatography meanwhile 116 active substances of pesticides by GC-MS/MS chromatography device were analyzed in sweet fennel seed. This study was conducted in years of 2012 and 2013 consecutively. Detectable pesticide residues have not been measured in the samples.

Keywords: Fennel, Pesticides, Residues, Chromatography, Burdur

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Introduction

Fennels (*Foeniculum vulgare* Mill. var. *Dulce*) are members of Apiaceae (Umbelliferae) family and their synonyms in Turkey are "rezene, raziyane, arapsaçı, irziyan and mayana". The origion of these spice plantsis reported to be Western Asia and Mediterranean Countries (Davis, 1982; Uzun et al., 2012). "Sweet fennels" (*Foeniculum vulgare* Mill. var. dulce) that are grown artificially as well, are spice plants (Baydar, 2005; Ceylan, 1997) found naturally in Northern, Southern and Western regions of Turkey (Davis, 1982) and are grown in limited areas of cities such as Bursa, Denizli, Gaziantep, Manisa and Antalya (Anonymous, 1999). "Hot fennels" are found wild in the Northern Anatolia region (Ordu and Trabzon) of Turkey (Zeybek, 1960; Baytop, 1999; Akgül, 1993).

While a great amount of fennel seeds was produced in Turkey, they are consumed in internal market, and the rest is being exported (Anonymous, 1999). Fennels shared about 4.2 % of Turkey's Medical Plant Export in 2008 and 1.915 tonnes of products were exported for 3.739.000 $ while 266 tonnes were imported for 386.000 $. 10 tonnes of fennels had been processed and as a response, 200 kg's of volatile oil had been obtained (Anonymous, 1999). Furthermore, rate of volatile oil obtained from fennels that are traded should not be under 1.5%. The 2% infusion obtained from seeds of fennels, whose leaves are known as wound healing and stems are known as diuretic, is known as gas removing and milk enhancer and was reported to be effective as spasms solvents and secreolytically effective (Baytop, 1999; Ernst, 2001). In addition, fennels have production license as herbal drugs by the Ministry of Health (Özçelikayet al., 1997). Fennels' launched medical forms are used for some diseases such as aches, swelling, gas pains and spasms of stomach and intestines and upper respiratory tract discharges (e.g: Flu) (Czygane, 1989; Weib, 1991).

Volatile oil and some other components produced by fennel seeds are used for food and medicine sectors and perfumes and cosmetics sectors (Baytop, 1994). There is a need for the determination of pesticide residues on fennel seeds and bringing these findings into the economy which serve a rewarding purpose for Turkey, as a developing country. Pesticides are used as protectors in agriculture and their overuse and misuse cause permanent toxic effect in nature. Their misuse cause acute and chronical diseases on people and cause environmental pollution. Although restriction shave taken place for pesticide use, harvest of premature products and introduction of these products to markets before the correct time resulted for pesticides to take place as residues in the food chain. Residues on earth are observed to pollute spring waters and drinking waters by the effects of rains and etc. When pesticides are misused, they may lead to many kinds of diseases for animals more than the plant itself, which pesticides are applied on, and this situation may go as far as death for animals. Active substances of pesticides are known to cause acute toxic and genotoxic effects on people (Anonymous, 2014). When analyzing the pesticide residues over the standards developed by Worlds Health Organization (WHO) and Food Agriculture Organization (FAO) highly sensitive analytical devices such as GC-MS/MS and LC-MS/MS are used (Ersoy et al., 2011a; Ersoy et al., 2011b). In this study, pesticide residues on fennel seeds that grown in Tefenni Town of Burdur city were analyzed.

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Materials and Methods

In this study, the seed materials obtained from fennel plants grown by a farmer in Teffeni town of Burdur city during 2012- 2013. Examples were gathered approximately 1 month later the harvest. As the purpose, 1 kg seed samples from 10 different sacks in the farmer's fennel stock were gathered and mixed. 15g of seed samples were studied for extractions for 3 times revealingly. Pesticides given in Figure 1 and 2 are searched in the examples of fennel seeds, which are the materials. All extraction studies and residue analysis of the samples were done at the Directorate of Food Control Laboratory of İzmir, connected The Food, Farming and Livestock Ministry.

All the solvents and chemicals (water, acetonitrile, methanol, formic acid, acetic acid, ammonium formate) used as mobile phases in sample extractions were chosen in accordance to a profound quality. Pesticide standards are prepared at least a 90% rate of purity. Extractions and clearance of the samples are generalized in accordance with AOAC (International Official Methods of Analysis) methods (AOAC, 2007).

Examples' Preparation for Analysis

15 g samples were homogenized in a mechanical shredder. Other similar samples of the same sample were put into same processes separately. Sample amounts that put into extraction were taken from these homogenized samples after weighing.

Extraction of Examples

Whole samples were homogenized with steel blenders by shredding and 5g of sample from the main sample were weighed and mixed with 10ml of water and 15ml of acetonitrile with 1% acetic acid and strongly shaked for 1 minute. Afterwards, 6 g of waterless magnesium sulfate ($MgSO_4$) and 1.5 g's of Sodium Acetate ($C_2H_3NaO_2.3H_2O$) was added into falcon tubes and after being shaked for 1 minute, centrifuged for 5 minutes at 4000 rpm rate. As the next step, 8 ml of samples from the previous examples' high phases were collected for the cleaning process and transported into 15 ml falcon tubes and mixed with 1.2 g of waterless $MgSO_4$ and 0.4 g's of PSA and centrifuged for 5 minutes at 4000 rpm rate, once again. Later, the high phase was transported into vials and kept in a freezer until the device evaluations. As the last injections into LC-MS/MS and GC-MS/MS devices were conducted and residue rates were determined. Chromatographically conditions of LC-MS/MS and GC-MS/MS devices are explained on Table 1 and Table 2 in detail.

3 Hydroxy carbofuran+Carbofuran; Acephate; Acetamiprid; Aclonifen; Aldicarb+sulfone+sulfoxide; Allaxydim Na; Amitraz+Metabolitleri ;Anilofos ;Atrazine ;Atrazine Desethyl; Azadirachtin; Azoxystrobin;Azyprotryn; Benalaxyl; Bendiocarb; Benfurocarb; Benomyl+Carbendazim; Bensulfuron methyl; Boscalid;Bromacil; Bromuconazole; Butocarboxim;Butylate ;Carbaryl;Carbosulfan;Carboxin;Carfentazone ethyl; Chlofentezine; Chlorbromuron; Chlorfenvinphos; Chloridazon; Chlormequat chloride; Chloroxuron;Chlorpyrifos;Chlorpyrifos methyl;Clodinafop propargyl ester;Clomazone;Coumachlor; Coumaphos;Cyanazine;Cycloate; Cymoxanil; Cyproconazole ;Cyprodinil; Demeton S methyl sulfoxide; Diazinon;Dibrom; Diclorvos (DDVP); Dicrotophos; Diethofencarb; Difenoconazole; Dimefox; Dimetha chlor; Dimethenamid;Dimethoate+Omethoate; Dimethomorph;Diniconazole; Dioxacarb; Diphenamid; Dipropetryn;Diuron; Dodine; Epoxiconazole; Ethion; Ethirimol; Ethoxyquin;Etrimfos;Famoxadone; Fena midone; Fenazaquin; Fenhexamid; Fenoxaprop; Fenoxycarb; Fenpiclonil; Fensulfothion; Fenuron; Fluquin conazole; Flurochloridone; Flutriafol;Fonofos; Formothion; Furathiocarb; Haloxyfop toxyethyl; Haloxyfop methyl; Heptenephos; Hexythiazox; Imazalil; Imazaquin;Imidocloprid; Indoxacarb ;Iodosulfuron methyl sodium; Iprodion; Iprovalicarb; Isopruton;Kresoxim methyl; Lenacil;Malaoxon;Malathion; Mecarbam;Mefenpyr diethyl;Mephosfolan;Mesosulfuron methyl; Metalaxyl+Metalaxyl; Metamitron; etazachlor; Metconazole; Methacrifos; Methamidophos; Methidathion; Methiocarb; Methomyl+Thiodicarb; Metolachlor; Metoxuron; Metribuzine; Metsulfuron methyl; Mevinphos;Molinate; Monocrotophos; Monolinuron; Myclobutanil;Nicosulfuron;Oxamyl;Paraoxon Ethyl;Parathion ethyl; Pencycuron;Phentoate; Phosalone; Phosmet;Phosphami don; Phoxim; Picloram; Pirimicarb; Pirimiphos ethyl; Pirimiphos methyl;Pirimisulfuron methyl; Prochloraz; Profenefos; Profoxydim; Promecarb; Prometryn; Propachlor; Propamocarb; Propaquizafop; Propazine; Propiconazole; Propoxur; Propyzamide;Proquinazid; Prosulfuron; Pymetrozine;Pyraclostrobin; Pyrazop hos;Pyrazosulfuron; Pyridaben; Pyridaphention; Pyriproxyfen; Quizalofop P ethyl;Rimsulfuron;Spinosad; Spiroxamin; Temefos; Tepraloxydim; Terbufos; Terbuthylazine; Terbutryn; Tetrachlorvinphos;Thiabendazole; Thia cloprid;Thiamethoxam;Thiophonate ethyl; Thiophonate methyl; Tolyfluanide; Tralkoxydim; Triadimefon+Triadimenol; Triallate; Triasulfuron; Triazophos; Trifloxystrobin; Triflumizole; Triflusulfuron methyl; Triticonazole; Tulfotep; Vamidothion; Vernolat; Zoxamide

Figure 1. Active substances examined in fennel seed examples on LC-MS/MS device (Detection Limit: 0,010-0,050 µg/kg)

1-3 Hexachlorobutadiene; Acetochlor; Alachlor; Aldrin+Dieldrin; Alpha BHC;Alpha+Beta+Endosulfan sülfat; Azinphos methyl; Azobenzene; Beta BHC; Bifenthrin; Bitertanol; Bromophos ethyl;Bromophos methyl; Bromopropylate; Bupirimate;Buprofezin;Cadusafos;Captan;Chlorfenapyr;Chlorfenson;Chloroneb; Chlorpropam; Chlorthal dimethyl; Chlorthalonil; Cis+Trans Chlordane; Cyfluthrin; Cypermehtrin + isomers; Cyromazine; Delta BHC; Deltamehtrine; Demeton-S-methyl; Desmethrine; Dialifos; Dichlofluanid;Dicofol; Dinobuton; Disulfoton;Disulfoton sulfone; Disulfoton sulfoxide; Ditalimphos; Endrin; Endrin aldehit; Endrin keton; EPN; Ethalfluralin; Ethiofencarb; Ethofumasate; Ethoprophos; Etoxazol; Fenamiphos; Fenarimol; Fenchlorfos; Fenitrothion; Fenpropathrin;Fenson; Fenthion; Fenvalerate+Esfenvalerate; Flamprop methyl;Flucythrinate; Fluotrimazole; Flusilazole; Folpet; Fuberidazole; Heptachlor + isomers; Hexachlorobenzene (HCB); Hexaconazole; Iodofenfos; Isazofos; Isodrin; Isopropalin, Lambda Cyhalothrin; Lindane (Gamma BHC);Linuron; Methoxychlor;Nuarimol;Ofurace;Oxidixyl; Oxyfluorfen; Pebutale; Penconazole; Pendimethalin; Pentachloroanilin; Pentanachlor; Permethrin; Phorate; Piperonyl butoxide;Procymidone;Propanil;Propargite; Prothiofos; Pyrimethanil; Pyrimidifen;Quinalphos; Quinomethionate; Quinoxyfen; Quintozene;Rabenzazole; Resmehtrin; Simazine;Sulprofos;Taufluvalinate;Tebuconazole;Tebufenpyrad; Tecnazene;Tefluthrine; Terbacil;Tetraconazole;Tetradifon; Tetrasul; Thiobencarb; Thiometon; Tolclofos Methyl; Total DDT; Trichlorfon; Trifluralin; Vinclozolin

Figure 2. Active substances examined in fennel seeds examples on GC-MS/MS device (Detection Limit: 0,010-0,050 µg/kg)

Table 1. Chromatographic Working Conditions of LC-MS/MS

LC	Agilent 1200/Binary		
MS/MS	Agilent 6410		
Mobile Phase	5 mM Amonium Formate &Water + Acetonitrile		
Mobile Phase Flow	0,6 ml/min		
Column	Eclipse XDB-C18; 3,5µm; 4,6*150mm		
Gradient	Time (min)	%A	%B
	0	85	15
	5	85	15
	20	10	90
	30	0	100
The Column Oven	25°C		
Injection Capacity	3 all		
MS Gas Temperature	350°C		
MS Gas Flow	12 l/min		
Nebulizer Pressure	40 psi		
Capillary	4000 V		
MS1 / MS2 Temperature	100°C / 100°C		
Bowl Vacuum	2,3 Torr		
High Vacuum	$8,79*10^{-6}$ Torr		
Delta EMV	400		

Table 2. Chromatographic Working Conditions of GC-MS/MS

Gas Chromatography	7890A			
Mass Detector	7000A			
Column	HP-5MS, 15 m, 250µm, 0.25µm			
Injection Volume	PTV Injektion, 5µl			
Carrier Gas, Flow	Helium (high purity)			
Mode of Operation	SIM			
PTV Temperature Program		Increase °C/min	Temperature (°C)	Time (min)
	Start		60	0,5
	Level 1	200	250	10
	Level 2	50	60	4
Oven Temperature Program		Increase °C/min	Temperature (°C)	Time (min)
	Start		50	0,75
	Level 1	25	150	0
	Level 2	3	200	0
	Level 3	8	280	15
Pressure	26,2 psi			
Vent Flow	100 ml/min			
Inlet	280°C			

Results and Discussion

The findings amount in the study are considered on an average of 3 repetitions of each example in accordance with the "Turkish Food Codex (TGK) Rescript of Maximum Residue Limits of Pesticides Permitted to be Found in Livestock (Official Newspaper: 21.01.2011-27822; Rescript No: 2011/2). Each residue limits of TGK for each pesticide example are given in the tables, separately. In the residue limits of fennel seed examples' examinations, where high-previsioned devices such as GC-MS/MS and LC-MS/MS were used, 186 active substances of pesticides in LC-MS/MS device and 116 active substances of pesticides at GC-MS/MS device were analyzed. In this study conducted during the years of 2012 and 2013, any analyzable residues were not observed in the examples of both years.

Ersoy et al., (2011a), in the study they conducted to analyze the pesticide residues on strawberries and table grape examples gathered from the markets of Konya area, found that, there is 34, 33 and 47 µg/kg (tolerate value 20 µg/kg) rate of Imazalil in the example of wet grapes; and there is 337 and 433 µg/kg Benomyl-Carbendazim (tolerate value 300 µg/kg) on 2 examples on wet grapes. In the study they conducted, in the wet grape examples on which they studied, the product without any pesticide residues is 38%, the product with 1 kind of pesticide residues is 10%, the product with 2 kind of pesticide residues is 20%, the product with 3 kind of pesticide residues is 10%, the product with 4 kinds of pesticide residues is 11%, the product with 5 pesticide residues is 9%, the product with 6-7 kinds of pesticide residues is 2% of all the examples. They determined the strawberry examples as 70% is without any pesticide residues and 30% is with pesticide residues, over-limit pesticide residues and with the use of forbidden chemicals.

Kurt et al. (2011), studied the spice and other spice kinds' exportation between the years of 1900 -2009. They reported that fennel have a role of 5% (27.7 million kg's) in exportation and 9% (3.46 million kg's) in spice importation of Turkey's spice trade.

Abou-Arabet et al., (1999), in the study they conducted, analyzed pesticide residues on 303 different examples of 20 different medicinal plants spices in Egypt and among these; hibiscus, dill, celery, tea, cumin and chamomile are taking place. Their findings showed that malathion is the superior pesticide residue on the analyzed examples. They reported that dimetohate rate on cumin and camomile examples passed the permitted limit (MPLs). On chamomile examples, lindane, aldrin, dieldrin, DDT, clordane and rates have passed the maximum limits (MPLs). They reported that when medical plants boiled in water, the residues cannot be analyzed; when the plants drown into water, the pesticide residues move into the liquid extract.

Abou-Arab (1999) in his study, study on the pesticide residues on tomatoes and tomato products in Egypt.He determined the average HCB, lindane, dieldrin, heptachlor epoxide and DDT sorts' levels in the following order 0.009, 0.003, 0.006, 0.008 and 0.083 mg/kg and he reported that he did not found any pesticide residues in ketchup and tomato paste. He suggested that tomatoes contain the highest level of HCB, lindane, dieldrin and DDT in their cuticular and sub-cuticular tissues. He underlined the importance of washing them with water and/or detergent solution to decrease the pesticide rate to the lowest level and cooking (As the tomato paste production process) them helps to eliminate the pesticide residues from tomatoes.

Santamaria et al., (1999), in their study they conducted in Bari (Italy), studied on 327 different examples from 26 different fresh vegetable kinds that contain nitrate and oxalate. Their findings suggested that the vegetables whose leaves are edible (celery, parsley, spinach, etc.); contain higher levels of nitrate than vegetables whose bulbs, tubers and shoots are edible. They found high level of oxalate in spinach and chard. They reported that the daily intake of nitrate is determined as 71 mg's by the national nutrition institute and as they reported, with chard and lettuce intake this value line is passed 30%.

Abou-Arab and Abou Donia (2001), in the study they conducted, studied pesticide residues, heavy metals and aflatoxin components on medical plants (peppermint, chamomile, fennel, cumin, etc) used on both children and grown ups. Examples were collected from different markets in Egypt. As a result, while malathion, dimethoate, and profenofos were found in high levels on most of the analysed examples; aldrin, dieldrin, chlordane and lindane were found in lowest levels. Chlorpyrifos, parathion, diazinon and endosulfan were not found in analysable levels.

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Conclusion

Tefenni/Burdur district is one of the most important regions in terms of organic production in our country. The use of pesticides in the region is reasonable. Our analysable residues were not observed in the examples of both years. This is good result for good agricultural practices. According to these results, organic farming can be applied in the region.

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