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Determination of Pesticide Residue Present in Cumin Plant (*Nigella orientalis* L.) with LC-MS/MS and GC-MS

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In this study, pesticide residues were investigated in black cumin (*Nigella orientalis* L.) seeds which grown in Turkey. GC-MS and LC-MS/MS analytical instruments are used in high precision, when determining residue limits. A total of 100 pesticide active ingredients in LC-MS/MS devices have been performed in *Nigella orientalis* L. seeds samples. In this plant, 103 pesticide active ingredients were analyzed in GC-MS. This study conducted in 2012 and 2013. Samples residues were not found in detectable levels for 2 years.

Keywords: Pesticide, Residue, Black cumin.

INTRODUCTION

Kinds of *Nigella* L. are originated from Mediterranean Region and Westerns Asia; they are members of *Ranunculaceae* family and they form 14 species [1]. They contain several important medicinal and aromatic species. It has been found that *N. sativa* and *N. damascena* L. Seeds have pharmacological effect [2,3].

Nigella orientalis is an annual plant and it can grow up to 30 cm; its flowers are yellow and its seeds are shaped as discs. They spread from North-East Mediterranean region, Caucasia and Crimeato Syria. Cumin has been used as a natural medicine in Middle-East and Far-East countries, over 2000 years now. They are grown in Southern Europe in a limited amount [4].

There are 15 species and 19 taxons in the flora of Turkey [1,5]. The plant is grown especially Afyon, Burdur, Isparta, Kütahya and Konya regions [6].

Its seeds are used as a flavour and the plant, itself, is grown as a foliage plant. Kokoska *et al.* [7] defined 18 compounds in *N. orientalis* L. seeds through their analyze of essential oils. They determined β-elemene (68.8 %), δ-cadinene (5.2 %) β-selinene (4.3) and α-selinene (3.3 %) in the highest rates. Noting that their study is the first on the field so far, as far as they have known, they claimed that, *N. orientalis* and *N. domascena* are more similar than *N. sativa*, in terms of essential oils.

Cumin seeds have been used as spice and nutrient protectors. The seed sand oil of the plant have potential medical features, which can be used in traditional medicine. Cumins theraupathic (healing) feature and its role in cure of diseases and effect on human body was examined in this compilation. Cumin has the effect of starting antioxidant system, which cleans oxidants. It shows anti-inflammatory property and also supports the defense system (immune) [8].

Wagner and Fransworth [9], suggested that there is 6.4 % water, 4 % ash, 32 % oil, 20.2 % crude protein, 6.6 % crude fiber and 37.4 % carbohydrate in cumin seeds. As an addition, cumin seeds contain B1, B2, B6 vitamins in small amounts and iron, calcium, magnesium, zinc and selenium, as they suggested [10]. Farag *et al.* [11] defined 62 metabolites in 6 *Nigella* species, also including *Nigella orientalis* and revealed 52 of these metabolites. They claimed that their study was the first to form a comparing metabolites profile among *Nigella* species.

Baser *et al.* [12] claimed that linoleic and oleic acids are observed as the main fatty acids, in their study, conducted with GC-MS and LC-MS/MS, to examine the chemical components of *Nigella* seeds.

Meziti *et al.* [13] suggested that cumin plants are traditionally used in the cure of asthma, coughing, bronchitis, head ache, rheumatism, high-fever, flu and eczema. The same researchers claimed that *Nigella* seeds are reported to have some biological activities, including antioxidant activity. They claimed that the chloroform extract and ethyl acetate are the highest rated phenolic components in *Nigella* seed sand ordinarily they contain 81.31 and 72.43 µg, by using Folin-Ciocalteu method.

Wajs and Kalemba [14] defined 30 components in *Nigella orientalis* L. seeds in Poland and claimed that 16 of these components are new in these species. Researchers suggested that the most known *Nigella* species are *N. sativa* (black cumin) and *N. damascena* L. (love in the mist) and naturally they spread through West-Europe, North-Africa and South west Asia. As an edition, they stated that researches on *N. orientalis* are rather limited.

Sirma and Kadioglu [15] studied weed species on wheat cultivation areas through their study in 2006. They defined 51 weed species belonging 20 families in total. They included *Nigella arvensis* into these species as well.

Tutukcu and Sagdic [16] defined linen, nigella, thyme, nettle and camomille a highly requested plants, their study to examine medical and their drugs, sold in herbal shops through the years 2008 to 2009 in Konya.

Kim *et al.* [17] in their study to investigate nematode activity in plant oils observed that 17.2 % nematode activity took place in the oil from India-originated *N. sativa*.

Pesticides are used as protectors in agriculture and their over/misuse lead toxic effects in nature. Their misuse cause acute and permanent health problems on people. Although restrictions on pesticide use are imposed, pesticides remain as residues on food chain as a result of reaping and marketing the products prematurely. Active substances of pesticides are known to cause acute toxic and geno toxic effects on people [1].

Highly sensitive analytical devices such as GC-MS and LC-MS/MS are used to define World Health Organization (WHO) and Food Agriculture Organization (FAO) international pesticide residue limits [18]. In this study, pesticide residues on Turkey originated Cumin (*Nigella orientalis* L.) seeds were investigated.

EXPERIMENTAL

In the study, the weeds obtained from *Nigella orientalis* L. plants grown by a farmer in Tefenni town of Burdur city in years of 2012-2013 are used as material. Cumin production is very prevalent around the region. Examples were gathered approximately 1 month later hence the harvest. As the purpose, 1 kg seed examples from 10 different sacks in the farmer's cumin stock were gathered and mixed together. 15 g of weed examples are studied for extractions 3 times repeatingly.

All the solvents and chemicals (water, acetonitrile, methanol, formic acid, acetic acid, ammonium formate) used as mobile phases in example extractions are chosen in accordance to a profound quality. Pesticides given in Tables 1 and 2 are searched in the examples of cumin seeds, which are the

TABLE-1
ACTIVE SUBSTANCES EXAMINED IN CUMIN SEEDS EXAMPLES ON GC-MS/MS DEVICE

Analyte	LOQ (μg/kg)	Analyte	LOQ (μg/kg)	Analyte	LOQ (μg/kg)
1-3 Hexachlorobutadiene	10	Ditalimphos	10	Oxyfluorfen	10
Total DDT	10	α,β-Endosulfan sulfate	50	Pebutale	10
Acetochlor	10	Endrin	10	Penconazole	10
Alachlor	10	Endrin aldehit	20	Pendimethalin	10
Aldrin+Dieldrin	10	Endrin keton	20	Pentachloroanilin	10
α-BHC	10	EPN	20	Pentanachlor	10
Azinphos methyl	10	Ethalfluralin	10	Permethrin	10
Azobenzene	15	Ethiofencarb	10	Phorate	10
β-BHC	10	Ethofumasate	10	Piperonyl butoxide	10
Bifenthrin	10	Ethoprophos	10	Procymidone	10
Bitertanol	10	Etoxazol	10	Propanil	10
Bromophos ethyl	10	Fenamiphos	10	Propargite	10
Bromophos methyl	15	Fenarimol	10	Prothiofos	10
Bromopropylate	10	Fenchlorfos	10	Pyrimethanil	10
Bupirimate	10	Fenitrothion	10	Pyrimidifen	10
Buprofezin	10	Fenpropathrin	10	Quinalphos	10
Cadusafos	10	Fenson	10	Quinomethionate	10
Captan	50	Fenthion	10	Quinoxyfen	10
Chlorfenapyr	25	Fenvalerate+Esfenvalerate	10	Quintozene	10
Chlorfenson	10	Flamprop methyl	10	Rabenzazole	10
Chloroneb	10	Flucythrinate	10	Resmehtrin	10
Chlorpropam	10	Fluotrimazole	10	Simazine	10
Chlorthal dimethyl	10	Flusilazole	10	Sulprofos	10
Chlorthalonil	20	Folpet	10	Taufluvalinate	10
cis + *trans*-Chlordane	10	Fuberidazole	10	Tebuconazole	10
Cyfluthrin	10	Heptachlor+izomerleri	10	Tebufenpyrad	10
Cypermehtrin+izomerleri	10	Hexachlorobenzene (HCB)	10	Tecnazene	10
Cyromazine	10	Hexaconazole	10	Tefluthrine	10
δ-BHC	10	Iodofenfos	15	Terbacil	10
Deltamehtrin	15	Isazofos	10	Tetraconazole	10
Demeton-S-methyl	10	Isodrin	10	Tetradifon	10
Desmethrine	10	Isopropalin	10	Tetrasul	10
Dialifos	10	λ-Cyhalothrin	10	Thiobencarb	10
Dichlofluanid	15	Lindane (γ-BHC)	10	Thiometon	10
Dicofol	10	Linuron	10	Tolclofos Methyl	10
Dinobuton	15	Methoxychlor	10	Trichlorfon	10
Disulfoton	10	Nuarimol	10	Trifluralin	10
Disulfoton sulfone	10	Ofurace	10	Vinclozolin	10
Disulfoton sulfoxide	10	Oxidixyl	10	–	–

TABLE-2
ACTIVE SUBSTANCES EXAMINED IN CUMIN SEED EXAMPLES ON LC-MS/MS DEVICE

Analyte	LOQ (µg/kg)	Analyte	LOQ (µg/kg)	Analyte	LOQ (µg/kg)
3-Hydroxy carbofuran + Carbofuran	10	Epoxiconazole	10	Parathion methyl	15
Acephate	50	Ethion	10	Pencycuron	10
Acetamiprid	10	Ethirimol	10	Phentoate	10
Aclonifen	10	Ethoxyquin	30	Phosalone	10
Aldicarb+sulfone+sulfoxide	50	Etrimfos	10	Phosmet	10
Allaxydim Na	10	Famoxadone	10	Phosphamidon	10
Amitraz+Metabolitleri	30	Fenamidone	20	Phoxim	10
Anilofos	10	Fenazaquin	10	Picloram	10
Atrazine	10	Fenhexamid	10	Pirimicarb	10
Atrazine Desethyl	10	Fenoxaprop P	10	Pirimiphos ethyl	10
Azadirachtin	20	Fenoxycarb	10	Pirimiphos methyl	10
Azoxystrobin	10	Fenpiclonil	10	Pirimisulfuron methyl	10
Azyprotryn	10	Fensulfothion	10	Prochloraz	10
Benalaxyl	10	Fenuron	10	Profenefos	10
Bendiocarb	10	Fluquinconazole	10	Profoxydim	10
Benfurocarb	10	Flurochloridone	10	Promecarb	10
Benomyl+Carbendazim	10	Flutriafol	10	Prometryn	10
Bensulfuron methyl	20	Fonofos	25	Propachlor	10
Boscalid	10	Formothion	20	Propamocarb	20
Bromacil	10	Furathiocarb	10	Propaquizafop	10
Bromuconazole	10	Haloxyfop etoxyethyl	10	Propazine	10
Butocarboxim	50	Haloxyfop methyl	10	Propiconazole	10
Butylate	30	Heptenephos	10	Propoxur	10
Carbaryl	10	Hexythiazox	10	Propyzamide	20
Carbosulfan	10	Imazalil	10	Proquinazid	10
Carboxin	10	Imazaquin	20	Prosulfuron	10
Carfentazone ethyl	10	Imidocloprid	10	Pymetrozine	20
Chlofentezine	10	Indoxacarb	10	Pyraclostrobin	10
Chlorbromuron	10	Iodosulfuron methyl sodium	10	Pyrazophos	10
Chlorfenvinphos	10	Iprodion	20	Pyrazosulfuron	10
Chloridazon	10	Iprovalicarb	10	Pyridaben	10
Chlormequat chloride	50	Isopruton	10	Pyridaphention	10
Chloroxuron	10	Kresoxim methyl	10	Pyriproxyfen	10
Chlorpyrifos	10	Lenacil	10	Quizalofop P ethyl	10
Chlorpyrifos methyl	10	Malaoxon	10	Rimsulfuron	10
Clodinafop propargyl ester	10	Malathion	10	Spinosad	20
Clomazone	10	Mecarbam	10	Spiroxamin	10
Coumachlor	20	Mefenpyr diethyl	10	Tulfotep	10
Coumaphos	10	Mephosfolan	10	Temefos	50
Cyanazine	10	Mesosulfuron methyl	10	Tepraloxydim	20
Cycloate	10	Metalaxyl+Metalaxyl M	10	Terbufos	50
Cymoxanil	25	Metamitron	10	Terbuthylazine	10
Cyproconazole	10	Metazachlor	10	Terbutryn	10
Cyprodinil	20	Metconazole	10	Tetrachlorvinphos	10
Demeton S methyl sulfoxide	30	Methacrifos	10	Thiabendazole	10
Diazinon	10	Methamidophos	10	Thiacloprid	10
Dibrom	10	Methidathion	20	Thiamethoxam	10
Diclorvos (DDVP)	10	Methiocarb	10	Thiophonate ethyl	10
Dicrotophos	10	Methomyl+Thiodicarb	10	Thiophonate methyl	10
Diethofencarb	10	Metolachlor	10	Tolyfluanide	30
Difenoconazole	10	Metoxuron	10	Tralkoxydim	20
Dimefox	10	Metribuzine	10	Triadimefon+Triadimenol	10
Dimethachlor	10	Metsulfuron methyl	10	Triallate	10
Dimethenamid	10	Mevinphos	10	Triasulfuron	10
Dimethoate+Omethoate	10	Molinate	10	Triazophos	10
Dimethomorph	10	Monocrotophos	10	Trifloxystrobin	10
Diniconazole	10	Monolinuron	20	Triflumizole	10
Dioxacarb	10	Myclobutanil	20	Triflusulfuron methyl	15
Diphenamid	10	Nicosulfuron	10	Triticonazole	10
Dipropetryn	10	Oxamyl	10	Vamidothion	10
Diuron	10	Paraoxon Ethyl	10	Vernolat	10
Dodine	50	Parathion ethyl	20	Zoxamide	10

materials. All extraction studies and residue analysis of the examples by The Directorate of Food Control Laboratory of Izmir, connected to The Food, Farming and Livestock Ministry.

Pesticide standards are prepared at least a 90 % rate of purity. Extractions and clearance of the examples are generalized in accordance with AOAC (International Official Methods of Analysis) methods [19].

Preparation for analysis: 15 g examples were homogenized in a mechanical shredders. Other similars of the same example were put into same processes separately. Example amounts that put into extraction were taken from these homogenized examples after weighing.

Extraction: Whole examples were homogenized with steel blenders by shredding and 5 g of analyze examples from the main example were weighed and mixed with 10 mL of water and 15 mL of acetonitrile with 1 % acetic acid and strongly shacked for 1 min. Afterwards, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate is added into falcon tubes and after being shaked for 1 min, centrifugated for 5 min at 4000 rpm. As the next step, 8 mL of examples 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 anhydrous $MgSO_4$ and 0.4 g of primary secondary amine (PSA) and centrifuged for 5 min at 4000 rpm rate, once again. Later, the high phase was transported into viales 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.

RESULTS AND DISCUSSION

In the study, the obtained average residue limits are considered as each sample has three replications, according to "Turkish Food Codex (TGK) communique of allowed maximum pesticide residue limits on food stuffs (Official Gazette: 21.01.2011-27822; Communique no: 2011/2). Each pesticide residue limits of TGK on the each sample are given one by one on tables. As the pesticide residue limits, defined by highly analytic devices such as GC-MS and LC-MS/MS 100 active pesticide substances in LC-MS/MS and 103 active pesticide substances in GC-MS were found in Cumin (*Nigella orientalis* L.) seeds. In the study, conducted through 2012 to 2013, any analyzable pesticide residues were not found in neither years' samples.

Mohamadin *et al.* [20] investigated *Nigella sativa* L. oil's effects on reducing PPr (widely used broad spectrum carbamate; insecticides) toxicity. They found by applying free radical scavenging method that, *Nigella sativa* L. oil reduces the PPr toxicity and reduced the oxidative stress zone in rats' brains.

Nagahban *et al.* [21] in their study, using *Artemisia sieberi* oil, found that, this oil sort had a potential to be used under the control of *C. maculatus*, *S. oryzae* and *T. castaneum.*

Rajendran and Sriranjini [22] investigated the use of organic products as fumigants for insect control in stocked products. There are several organic products developed to form a toxic effect against stored grain pests [23-25]; especially the ones in vegetable oil formation [26,27]. Tunc *et al.* [28] claimed that, as an alternative for traditional fumigants, vegetable tallow extracted from aromatic plants have been investigated widely and toxic effects of insecticides for stored products are taken care for last ten years. Most of these studies investigate the effects of vegetable oil against these livings, who are in larvae and adulthood periods. They studied vegetable oil from 5 different plant species, which have an effect on these pests during their egg phase.

Shaaya *et al.* [29] in their study, questioned the fumigant toxicity of vegetable oil from five different aromatic plant species, against pests, effecting stored products. As *Tribolium castaneum* (L.), *Sitophilus oryzae* L., *Rhyzopertha dominica* (F.) and *Oryzae phksurinamensis* (L.) compared, *Tribolium castaneum* (L.) was reported to be more stable than the others.

Abou-Arab [30] had a study on pesticide residues, heavy metals and aflatoxin compounds on medical plants (mint, camomille, anise, cumin), used for both babies and adults. Samples were gathered from different markets of Egypt. As a result, while malathion, dimethoate and profenofos were observed to be in high rates; aldrin, dieldrin, chlordane and lindane were observed to be in low rates. Chlorpyrifos, parathion, diazinon and endosulfan were not observed to be in analyzable rates.

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