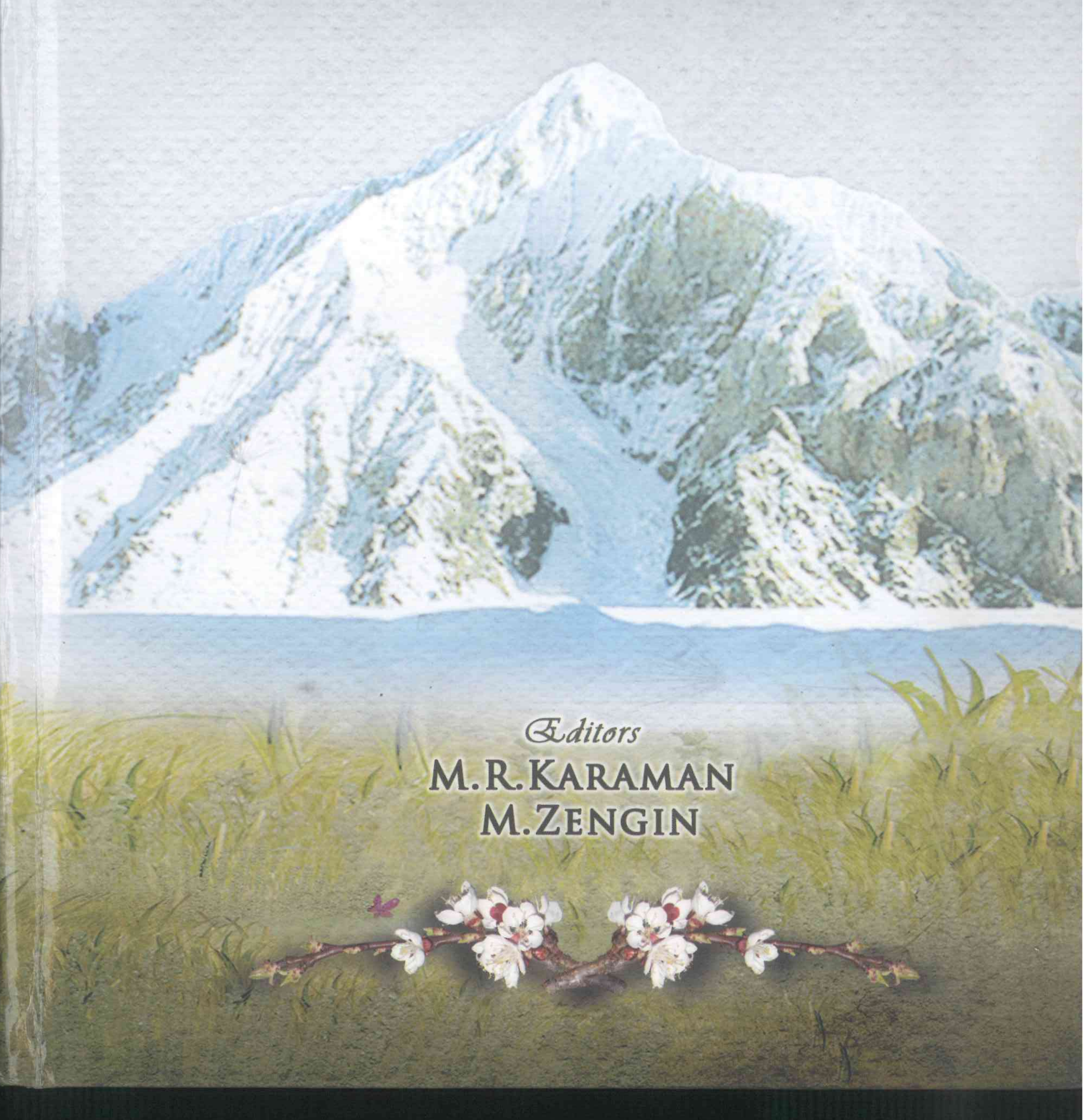
SOIL-WATER JOURNAL

2013 VOLUME 2 NUMBER 2 (2) ISSN: 2146-7072



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SOIL-WATER JOURNAL

Vol 2 (2013) Number 2 (2)

Special Issue for AGRICASIA'2013

'1st Central Asia Congress on Modern Agricultural Techniques and Plant Nutrition'

01-03 October 2013

Kyrgyz-Turkish Manas University (KTMU)
Cengiz Aytmatov Campus, Bishkek, Kyrgyzstan

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A New Approach in Pesticide Analysis in Soil Using Modified QuEChERS Method

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Abstract: A modified quick, easy, cheap, efficient, rugged and safe method (QuEChERS) coupled to gas chromatography with nitrogen phosphorus and electron capture detector (GC–NPD/ECD) was developed for rapid extraction and simultaneous determination of 45 multiclass pesticides in soil. The extraction solvent (acetone, acetonitrile, dichloromethane and ethyl acetate), time of extraction (1–30 minutes) and purifying agent (neutral alumina, primary secondary amine, graphite carbon black and florisil) for QuEChERS extraction were optimized. The method was validated in terms of linearity, selectivity, reproducibility, stability and recovery. The limits of detection (LODs) of the developed method for all investigated pesticides ranged from 0.003–0.03 mg kg^{-1} and limits of quantification (LOQs) from 0.005–0.05 mg kg^{-1}. The satisfactory data demonstrated the good reproducibility and stability of the method with relative standard deviations (RSDs) lower than 15%. Mean recoveries for spiked soil samples at each of the three spiking levels between 0.005–2.5 mg kg^{-1} ranged from 70–120%. The results of validation show that the method described here is suitable for the analysis of determined 45 multiclass pesticides in soil and were found to be practicable in the routine residue analysis.

Keywords: Gas chromatography, GC–NPD/ECD, pesticide residues, QuEChERS, soil

1. Introduction

Analytical methods for qualitative and quantitative analysis of pesticide residues are still developed and improved. In recent years, researchers are looking for a simple, effective, and inexpensive way to extract and clean pesticide residues from the many varied sample matrices that they worked with routinely. Traditional sample preparation methods (liquid-liquid extraction, Soxhlet extraction, etc.) are laborious, time consuming, expensive, requires large amounts of organic solvents and usually involve many steps, leading to loss of some analyte quantity. Modern sample preparation procedures have been developed to overcome the drawbacks of the traditional approaches, such as QuEChERS (quick, easy, cheap, efficient, rugged and safe).

The QuEChERS method is one of the most popular sample preparation method for multiresidue pesticide analysis, because is a fast, simple, and effective alternative to conventional procedures. There is an abundance of the QuEChERS applications for pesticides determination in different matrices. Thus, for example, QuEChERS provides satisfactory results for determination of pesticides in fruits (Camino-Sanchez et al., 2010; Cieślik et al., 2011), in vegetables (Wang et al., 2010; Li et al., 2013), in crops (Marchins et al, 2012), in rice (Thanh et al., 2007), in milk (In-Seek et al., 2012) and olive oil (Garcia-Reyes et al., 2007). However, there is only few reports in the literature about QuChERS application as a sample preparation method of soil samples and they are fragmented.

The paper presents a modification of the QuEChERS method involving the use of different extraction solvents, time of extraction and purifying agent. The present work discusses the possibility of using optimized QuEChERS for qualitative and quantitative determination of pesticide residues from different chemical groups in soil samples.

2. Materials and Methods

Acetone, acetonitrile, dichloromethane and ethyl acetate for pesticides residue analysis were provided by J.T. Baker (Deventer, Holland) and Florisil (60–100 mesh). Primary secondary amine and graphite carbon black were purchased from Fluka (Seelze-Hannover, Germany). Neutral aluminum oxide (0.063–0.200 mm) were obtained from Merck (Darmstadt, Germany). Anhydrous magnesium sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was produced using an Elix 3 Advantage system (Millipore, Molsheim, France). The pesticide standards were purchased from Dr. Ehrenstorfer Laboratory (Augsburg, Germany), the purities ≥ 95%. Each stock solution at various concentrations was prepared in acetone and stored at 4 °C for further dilution. Multicompound standard working solution (containing forty five active substances) were prepared by dissolving appropriate volume of each stock solutions in an *n*-hexane/acetone (9:1, v/v) mixture. The stock and working solutions were stored in completely filled vials closed with parafilm at -20 °C until the time of analysis.

Blank soil samples were collected from the vincinty of Bialystok (loamy sand, organic matter content < 2%) and were used for fortification experiments. Spiked samples were prepared by adding an appropriate volume of spiking solution to exactly weighed portions of air-dried and sieved soil sample (10 g) to produce final concentrations between 0.005–2.5 mg kg^{-1} and left for 1 h (to allow pesticide absorption by the sample). 10 g of sample was accurately weighed into a 50 mL glass centrifuge tube. Then, 2 mL of deionized water was added on the soil sample in order to make pores in the sample more accessible to the extraction solvent and the mixture was gently shaken for several seconds by hand. Then, 10 mL extraction solvent of acetonitrile was rapidly added into the sample and 4g of anhydrous magnesium sulfate followed by vortex mixing. Then the mixed solution was extracted by centrifugation at 5000 rpm for 5.0 min. 5 mL the supernatant was transferred into a new centrifuge tube with 500 mg florisil as purifying agent. After being shaken by vortex, the new mixed solution was centrifuged at 5000 rpm for 1 min. After centrifugation, 1 mL the supernatant was loaded into sample bottles for GC analysis.

Pesticide analysis was performed using an Agilent 7890 A gas chromatograph (Santa Clara, CA, USA) equipped with an automatic split-splitless injector Model HP 7683, a ^{63}Ni micro-electron capture detector (μECD) and a nitrogen phosphorous detector (NPD). Chemstation chromatography manager data acquisition and processing system (Hewlett-Packard, version A.10.2) was used. Chromatographic separation was performed on an Agilent HP-5 column (30 m, 0.32 mm I.D., 0.25 μm film thickness) (Little Falls, DE, USA).

3. Results and Discussion

3.1. Optimization

Several parameters were studied to optimize the performance of the extraction method, such as the extraction solvent, the extraction time and purifying agent Conditions for the best extraction efficiency were used for the rest of the study. The selection of extraction solvent was beneficial to improve process efficiency. Acetone, acetonitrile, dichloromethane and ethyl acetate were tested, and recoveries of the 45 pesticides were selected as the optimization indexes. The results showed that the extraction efficiencies from acetonitrile, dichloromethane and ethyl acetate were significantly better than those from acetone (recoveries < 60% for aldrin, chlorothalonil, dichlofuanid, fenhexamid, iprodione, primiphos-methyl and trifluralin) (Table 1). The extraction efficiencies acetonitrile, dichloromethane and ethyl acetate were similar for most pesticides, which ranged from 70% to 118%. Through the comparison of the recoveries, the extracts of soil from dichloromethane and ethyl acetate contained fewer interfering substances than those from acetonitrile. So, acetonitrile was finally chosen as the extracting solvent for soil with satisfactory recoveries.

The influence of extraction time was evaluated testing 1, 5, 15 and 10 min, followed by centrifugation. During experiments observed that recoveries improved when extraction time increased from 1 to 5 min. Further increase of the extraction time resulted in no significant improvement on analyte recoveries. The lowest recoveries for the most compounds were obtained using an extraction time of 1 min. Mean recoveries obtained using 15 and 30 min were lower than those obtained after 5 min. Comparing this, five minutes was the efficient extraction time of all compounds and was chosen as the optimum time. This value is a short time. Sample clean-up was necessary for the removal of polar co-extracted substances. Three kinds of sorbents including primary secondary amine (PSA), graphite carbon black (GCB) and florisil were tested for the investigated pesticides from soil samples. The recoveries of 45 pesticides in these sorbents (500 mg) were compared.

Table 1. Solvent effect on the pesticide residues extraction efficiency, 0.1 mg kg^{-1}

No.	Active substance	Substance group	de	Recovery [%] ± Relative standard deviation [%]			
				Acetone	Acetonitrile	Dichloromethane	Ethyl acetate
1.	Benalaxyl	Acylalanine	F	105±8	104±8	102±2	102±4
2.	Mepanipyrim	Anilinopyrimidine	F	97±6	98±10	101±2	103±3
3.	Pirimicarb	Carbamate	I	90±6	94±9	98±10	91±7
4.	Boscalid	Carboxamide	F	83±10	96±5	97±9	89±3
5.	HCB	Chlorinated aromatic hydrocarbon	F	101±9	96±4	107±13	93±11
6.	Dieldrin	Chlorinated hydrocarbon	I	80±4	87±13	86±14	77±11
7.	Propachlor	Chloroacetamide	H	75±7	110±9	105±10	104±7
8.	Chlorothalonil	Chloronitrile	F	59±9	85±11	95±15	100±5
9.	Aldrin	Cyclodiene	I	57±2	79±6	87±7	98±9
10.	Iprodione	Dicarboximide	F	58±8	101±9	115±15	99±6
11.	Procymidone		F	104±4	113±3	108±5	106±4
12.	Pendimethalin	Dinitroaniline	H	99±9	103±6	79±8	102±4
13.	Trifluralin		H	58±2	101±2	99±3	100±7
14.	HCH-alpha	Organochlorine	I	96±4	93±3	92±6	87±10
15.	HCH-beta		I	94±9	93±2	97±6	88±9
16.	Dicofol		A	107±7	105±15	114±4	118±1
17.	Endrin		I	82±1	89±3	87±2	85±8
18.	HCH-gamma (lindane)		I, A	102±10	103±11	101±3	109±8
19.	Endosulfan-alpha		I, A	84±14	99±8	95±5	92±12
20.	Endosulfan-beta		I, A	82±6	99±3	93±9	83±5
21.	Endosulfan-sulfate		I, A	88±6	94±14	96±2	91±14
22.	Heptachlor		I	92±5	97±4	99±4	96±3
23.	Heptachlor-epoxide		I	89±2	101±7	107±2	91±15
24.	Methoxychlor (DMDT)		I	116±2	114±9	113±3	117±8
25.	op'-DDT		I	76±2	77±5	70±9	72±3
26.	pp'-DDD		I	86±2	94±2	95±2	90±7
27.	pp'-DDE		I	91±14	95±8	91±9	96±6

Table 1. Continued

No.	Active substance	Substance group	de	Recovery [%] ± Relative standard deviation [%]			
				Acetone	Acetonitrile	Dichloromethane	Ethyl acetate
28.	pp'-DDT		I	71±9	75±12	70±6	73±7
29.	Chlorpyrifos	Organophosphate	I	97±8	100±10	101±8	98±6
30.	Chlorpyrifos-methyl		I, A	105±7	107±8	103±5	100±5
31.	Diazinon		I, A	73±6	95±5	78±13	112±11
32.	Pirimiphos-ethyl		I, A	74±4	71±7	82±3	76±2
33.	Pirimiphos-methyl		I, A	56±7	104±2	111±1	103±3
34.	Cypermethrin	Pyrethroid	I	73±15	85±12	81±5	86±11
35.	Deltamethrin		I	87±9	93±5	94±8	89±8
36.	Azoxystrobin	Strobilurin	F	91±6	105±5	107±7	97±4
37.	Trifloxystrobin		F	78±2	83±6	104±6	85±8
38.	Dichlofluanid	Sulphamide	F	51±3	80±5	91±4	109±7
39.	Tolylfluanid		F	67±4	93±3	112±5	89±6
40.	Cyproconazole	Triazole	F	97±8	103±6	101±3	90±8
41.	Difenoconazole		F	75±7	72±3	77±8	72±4
42.	Fenbuconazole		F	90±5	91±7	98±4	95±5
43.	Hexaconazole		F	100±9	104±5	92±8	101±3
44.	Tebuconazole		F	100±9	103±2	110±7	109±3
45.	Fenazaquin	Unclassified	A, I	102±5	100±2	102±3	104±3

A-acaricide, F-fungicide, H-herbicide, I-insecticide

The recoveries of PSA (a traditional sorbent) fluctuated due to matrix interference in the GC–NPD/ECD determination. The recoveries of GCB were low < 40%, which might result from the strong adsorption of GCB to target pesticides. While, the recoveries of 45 pesticides using florisil as a clean-up adsorbent were satisfactory < 70% and minimized interfering peaks from the matrix. The optimum QuEChERS conditions were the following: 10 g of soil sample, 10 mL of acetonitrile as a extracting solvent, extraction performed by centrifugation during 5 min, followed by clean-up step with 500 mg of florisil prior to the final determination by gas chromatography GC-NPD/ECD.

3.2. Validation

The established optimized QuChERS method was validated in terms of linearity, limit of detection (LOD) and limit of quantification (LOQ), selectivity, reproducibility, and recovery in accordance to EURACHEM/CITAC Guide (1998). Calibration standards were prepared in matrix solution (by adding respective spiking solutions to blank matrix of soil) to produce final concentration between 0.005-2.5 mg kg^{-1}.

Correlation coefficients were all greater than 0.996 for 45 pesticides. LODs of the developed method for all investigated pesticides ranged from 0.003–0.03 mg kg^{-1} and LOQs from 0.005–0.05 mg kg^{-1}. For recovery studies, the pesticide-free soil samples were fortified with all pesticides at three spiking levels: the first ranging between 0.005 and 0.05 mg kg^{-1}, the second at 0.05–0.5 mg kg^{-1} and the third within 0.25-2.5 mg kg^{-1}. Analyses were carried out in triplicate. The average recoveries for all analytes ranged from 71% to 114%. The satisfactory data demonstrated the good reproducibility and stability of the method with relative standard deviations (RSDs) ranging from 1% to 15%. The above-described results showed that the developed method was precise, accurate and sensitive enough for simultaneously quantitative determination of 45 pesticides in soil.

3.3. Field samples

The analytical methodology developed was finally applied to analysis of five soil samples collected from private customers from Podlasie region (loamy sand , organic matter content 3–4%). In one samples p,p'-DDE (0.05 mg kg^{-1}) and p,p'-DDD (0.005 mg kg^{-1}) were detected (Fig. 1). It was obtained the rest samples were no pesticide residues were found.

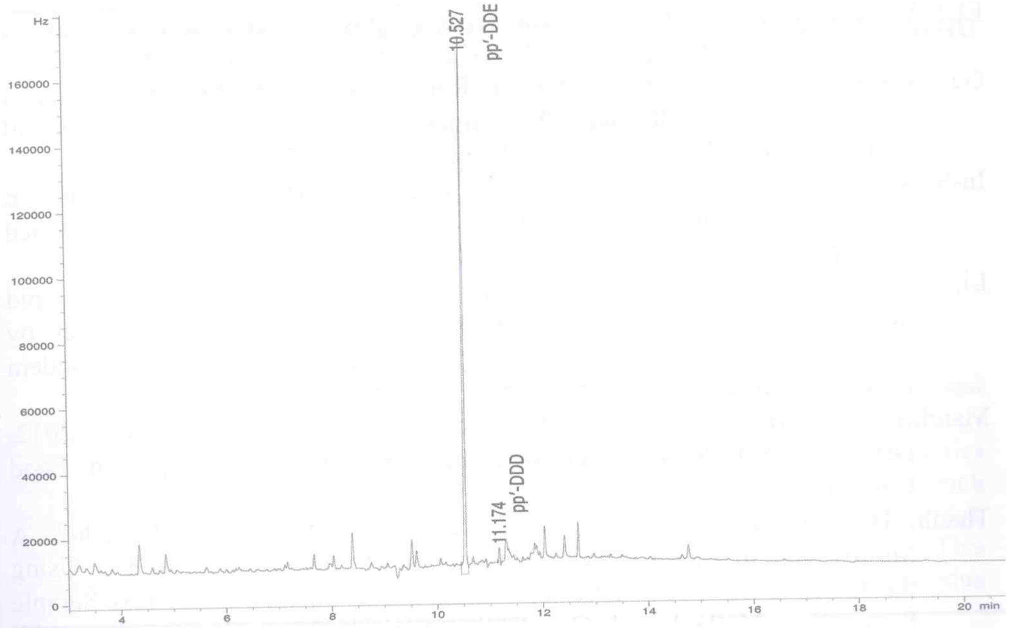

Fig. 1. Chromatogram of the real soil samples (pp'-DDE – 0.05 mg kg^{-1} and pp'-DDD – 0.005 mg kg^{-1})

Conclusion

In this article, a modified QuEChERS method was introduced for rapid extraction of 45 pesticides in soil by optimizing extraction solvent, extraction time and purifying agent. The proposed QuEChERS procedure was employed to detect a broad range of pesticides and achieved acceptable results. It was successfully applied to the sample preparation of field soil samples. QuEChERS method presents important advantages due to its simplicity, little-needed solvent and a little time-consumption.

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Comparison of Pesticide Residues in Raspberries and Strawberries from Organic and Conventional Farming in Poland

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Abstract: The aim of the present work was to compare organic and conventional samples of strawberry and raspberry in terms of pesticide residues. A total of 294 samples, including ecological: strawberries (100 samples) and raspberries (40), conventional: strawberries (114) and raspberries (40), were analyzed, in which 20 different pesticides were determined (4 insecticides, 15 fungicides and 1 herbicide). The most occurrence pesticides were boscalid, dithiocarbamates and fenhexamid. This research has demonstrated that organic fruit have smaller amounts of pesticide residue than produce that was grown conventionally.

Keywords: conventional, fruit, multimethods, organic, pesticide residues,

1. Introduction

The difference between organic and conventional food is the way how the food has been produced and processed. For instance, the use of fertilizers and pesticides is restricted in organic production. Organic food is defined as the product of a farming system, which avoids the use of man-made fertilisers, pesticides, growth regulators. Instead, the agricultural systems rely on crop rotation, animal and plant manures, some hand weeding and biological pest control. "Organic" farming is the description used in English-speaking countries, whereas in other markets "Bio" or "Eco" are the more usual product descriptions. All food cold as 'organic' in Poland must be produced according to European laws on organic production (Council Regulation). Although organically produced foods cannot be defined as pesticide free, the direct use of other than "traditional" agricultural chemicals is prohibited. Few studies have been done to determine the health benefits between organic and conventional foods, making it difficult to know the differences. However, a few studies have reported that organic produce has higher levels of Vitamin C, certain minerals and antioxidants (Lairon, 2010). But the differences are so small that they probably have no effect on overall health. There is no evidence that organically produced food is any safer, more nutritious than its conventionally produced counterpart.

Organic products in Poland are approximately 200% more expensive than conventional food (globally, this difference is equal to only 20%). This results from higher costs of cultivation and breeding but also from the fact that Poland ranks

34th in the world as a producer of organic food (Australia is first, and in Europe, Austria). Poland produces and exports the largest amount of strawberries originating from conventional production in Europe. On average, annual production amounts to approx. 250 thousand tons, and Poland's involvement in deliveries of this fruit to the EU market constituted 76.7% of frozen strawberries and 44.9% of fresh strawberries (mainly Senga Sengana). Raspberries are the second economically important crop in Poland, and for years, Poland has been among the world leaders in the production of this fruit. Raspberries are grown on over 10 thousand hectares, which gives 40-60 thousand tons of fruit production per annum. Berry fruits are very susceptible to fungal diseases, the occurrence of which is often dependent on the weather. This is why fungicides are commonly used to protect strawberries and raspberries against: *Botrytis cinerea* Pers., *Mycosphaerella fragariae*, *Sphaerotheca macularis* Wallr., *Phytophthora cactorum*, *Didymella applanata*. *Botrytis cinerea* Pers., most frequently occurring in strawberry plantations, can cause a reduction of crops by 20-60% while worsening the quality of the fruit at the same time, by itself. Smaller, but still noticeable, losses affecting the quantity and quality of a crop are caused by the pests: *Anthonomus rubi* Hbst., *Byturus tomentosus*, *Tetranychus urticae* Aderch., *Lasioptera rubi teneotarsonemus pallidus* Zimm. Besides diseases and pests, weeds can also be a decisive factor to strawberry crops. The aim of the present work was to compare organic and conventional management on a strawberry and raspberry orchard in terms of pesticide residues.

2. Materials and Methods

Pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Pesticide standard stock solutions and standard working solutions (purity higher than 95%) of various concentrations were prepared in acetone or hexane/acetone (9:1) and stored in dark below 4°C. All reagents used were analytical grade. Acetonitrile, Acetone, *n*-hexane, diethyl ether, toluene, dichloromethane and methanol for pesticides residue analysis were provided by J.T. Baker (Deventer, Holland), as well as florisil (60-100 mesh) and phosphate buffer pH=8. Hydrochloric acid, sodium hydroxide, potassium hydroxide, zinc acetate dehydrate grade, anhydrous sodium acetate and sulfate, anhydrous tin (II) chloride, ammonium Fe(III) sulphate were purchased from POCH (Gliwice, Poland). Silica gel (230–400 mesh), N,N-dimethyl-1,4-phenylenediammonium dichloride, sodium sulfide nonahydrate and celite were obtained from Merck (Darmstadt, Germany).

2.1. Samples

A total of 294 samples of berry fruit (40 samples of conventional strawberries, 100 BIO strawberries and 114 samples of conventional raspberries and 40 BIO raspberries) were collected in 2009 – 2012 from the north-eastern Poland. Some of the BIO samples were collected by inspectors of bodies certifying

organic farms (40%) and others came from individual clients and processing establishments producing BIO and conventional food.

2.2. Determination of 180 pesticide residues using GC/NP/EC

A 2 g of homogenized sample was put in a mortar with 4 g solid support silica gel. All was manually blended and transferred to a glass column with anhydrous sodium sulfate (5.0 g) and silica gel (2.5 g). The analytes were eluted using 15 mL hexane/acetone (8:2, v/v) and 15 mL hexane/diethyl ether/acetone (1:2:2, v/v/v). The extract was evaporated to dryness using rotary vacuum evaporator at a temperature of about 40 °C. Then the eluate was re-dissolved using 2 mL of hexane/acetone (9:1, v/v). The final solution was transferred into a GC vessel and placed to the rack of the autosampler. GC analysis was performed with a gas chromatograph model 7890A equipped with an automatic split-splitless injector and two selective detectors: electron capture (EC) and nitrogen-phosphorous (NP), fused silica capillary column 5%-phenyl-methylpolysiloxane; 30 m×0.32 mm, 0.50 µm (Agilent, Waldbronn, Germany). The injection port and detector (ECD/NPD) temperatures were 210 and 300°C, respectively, with helium as carrier gas at a flow rate of 3 mL/min. Nitrogen was used as a make up gas at a flow-rate of 57 mL/min (ECD) and 8 mL/min (NPD); hydrogen 3.0 mL/min, air 60 mL/min (NPD). For oven – initial temperature: 120°C increase to 190°C at 16°C /min, then to 230°C at 8°C /min and finally to 285°C at 18°C /min and hold 10 min (ECD and NPD).

2.3. Determination of carbendazim residues using HPLC

A 20 g of sample was homogenized with 150 mL acetone for 5 min. Then 2.5 g of celite was added to the extract and filtered. Final filtrate was evaporated and 20 mL of solution was applied to a ChemElut cartridge containing diatomaceous earth and pesticides were eluted with dichloromethane. The organic solvent was evaporated to dryness and dissolved in 2 mL volume of a mixture of acetonitrile/water (2:8, v/v). The final solution was put into a HPLC (liquid chromatograph Waters Alliance (Milford, MA, USA) model 2695 with photodiode at 285 nm and fluorescent detectors (λex=285 nm, λem=315 nm), Supelcosil LC-18, 5 µm, 250 mmx4.6 mm). The mobile phase was acetonitrile/phosphate buffer pH=8, a flow rate of 0.8 mL/min with a gradient composition, consisting of 20% (v/v) acetonitrile for 2 min, a linear increase over 13 min to 50% acetonitrile, then an increase to 80% acetonitrile over 5 min and finally a decrease at 20% acetonitrile over 5 min.

2.4. Determination of dithiocarbamates using spectrophotometry

Dithiocarbamate residues, a group (mancozeb, maneb, methiram, propineb, thiram, ziram), expressed as carbon disulphide, were determined by a modified colorimetric method. A 50 g of sample was heated for 45 minutes (80°C) with 60 mL of HCl and tin (II) chloride to release carbon disulphide from CS_2 in an alkaline pH. Ditiocarbamates decomposed with emission of CS_2 was separated and collected in a methanolic solution of potassium hydroxide where CS_2 formed potassium xantogenate which was next heated with zinc acetate to obtain zinc sulfide. This compound in an acidic medium released hydrogen sulfide which formed with N,N-dimethyl-1,4-phenylenediammonium dichloride and in presence of iron ions Fe (III) methylene blue. Finally, the quantity of formed was estimated by measure of absorbance at 662 nm wavelength on spectrophotometer (Helios Delta VIS). The absorbance was calculated into concentration and results were expressed in mg CS_2/kg.

2.5. Quality check and method validation

The laboratory successfully participated in the proficiency testing schemes organized and run by the European Commission (University of Almeria) and by the Food Analysis Performance Assessment Scheme (FAPAS; Central Science Laboratory in York). All of the analyses were conducted with the use of an accredited method by the Polish Centre of Accreditation (PCA). The validation of the analytical methods was carried out in accordance with European Commission (EC) guidelines (Sanco, 2011). The validation studies were performed using pesticide-free fruit samples, previously analyzed. Calibration standards were prepared in matrix solution to produce a final concentration of three spiking levels (0.005 to 0.05, 0.05–0.5 and 0.25–2.5 $mg \cdot kg^{-1}$). Method accuracy and precision were evaluated by performing recovery studies and are expressed as relative standard deviation (RSD, %) and mean recovery, respectively. Repeatability was calculated for five days using five replicates for each level of three different concentration levels. The sensitivity was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ) of the assay.

3. Results and Discussion

An analysis of pesticide residue data was performed to describe and quantify differences between organically grown and non-organic fresh fruits. The results of studies are presented in Table 1. The presence of residues of chemical crop protection products was stated in 13.6% of the samples (16) among the 140 studied samples of strawberries and raspberries from organic farms. Strawberry samples predominantly contained residues (17%). Multi-residue samples were noted. In 4.3% of all BIO samples, residue from one substance was detected, in 1.4% - two substances, and in 0.4% - 3 substances. A total of 14 active substances were detected from among the 180 pesticides being searched for, including 4 insecticides, 9 fungicides, and 1 herbicide. The presence of residues of chemical crop protection products was stated in 63% of the samples (97) among the 154 studied samples of strawberries and raspberries from conventional farms. 49% of these were below the permissible MRL limit, and 17% were above MRL. Residues of 14 out of the 180 substances being searched for were detected, including 4 insecticides and 10 fungicides.

Table 1. Pesticide residues detected in tested domestic organic and conventional fruit in 2009-2012

Fruit	Pesticide	Type	N° of samples	Range concentration ($mg \cdot kg^{-1}$)	Sum ($mg \cdot kg^{-1}$)	Mean ($mg \cdot kg^{-1}$)	MRL ($mg \cdot kg^{-1}$)
STRAWBERRY E	biphentrin	I/P	2	0.030-0.030	0.060	0.030	-
	boscalid	F	2	0.011-0.090	0.100	0.050	-
	chlorpyrifos	IF	2	0.001-0.012	0.011	0.0055	-
	cyprodinil	F	1	0.010	0.010	0.010	-
	cyfluthrin	I	2	0.021-0.032	0.050	0.025	-
	cypermethrin	I/P	2	0.02-0.052	0.070	0.035	-
	DTC	F	2	0.054-0.093	0.140	0.070	-
	fenhexamid	F	1	0.001	0.001	0.001	-
	folpet	F	1	0.035	0.030	0.030	-
	lenacil	H	3	0.013-0.032	0.060	0.020	-
	pirymethanil	F	3	0.034-0.045	0.100	0.033	-
	pyraclostrobin	F	4	0.033-0.086	0.190	0.047	-
	tetraconazole	F	2	0.033-0.744	0.100	0.050	-
	tebuconazole	F	1	0.030	0.030	0.030	
STRAWBERRY C	biphentrin	I/P	3	0.030-0.050	0.130	0.043	0.050
	boscalid	F	13	0.022-0.142	1.100	0.0851	10.00
	chlorpyrifos	IF	1	0.008	0.008	0.008	0.020
	cyprodinil	F	9	0.045-0.126	0.850	0.094	5.000
	DTC	F	4	0.955-0.123	1.38	0.345	10.00
	fenhexamid	F	4	0.024-0.035	0.09	0.023	5.000
	fludioxonil	F	7	0.010-0.133	0.36	0.051	3.000
	folpet	F	1	0.050	0.05	0.05	3.000
	iprodione	F	6	0.070-0.100	0.82	0.137	15.00
	carbendazime	F	2	0.022-0.034	0.05	0.025	0.100
	pirymethanil	F	1	0.033	0.03	0.030	5.000
	pyraclostrobin	F	4	0.155-0.255	0.68	0.170	5.000
	trifloxystrobin	F	1	0.030	0.03	0.030	5.000
RASPBERRY E	DTC	F	1	0.030	0.030	0.030	-

Table 1. Continued

RASPBERRY C	Boscalid	F	26	0.030-0.250	3.5	0.1346	10.00
	chlorpyrifos	I	4	0.020.-0.500	0.280	0.070	0.050
	Cyprodinil	F	19	0.020-0.300	3.800	0.200	10.00
	DTC	F	34	0.030-0.175	3.900	0.115	0.050
	Fenazaquin	I	2	0.010	0.020	0.010	0.010
	Fenhexamid	F	38	0.025-0.372	5.200	0.136	10.00
	Fludioxonil	F	9	0.342-0.560	2.900	0.322	5.000
	Folpet	F	12	0.034-0.500	2.400	0.200	3.000
	Iprodione	F	16	0.032-0.153	1.800	0.113	10.00
	carbendazime	F	2	0.034-0.082	0.110	0.055	0.100
	pirymethanil	F	32	0.033-0.672	3.600	0.113	10.00
	procymidone	F	12	0.010-0.152	0.980	0.082	0.020
	pyraclostrobin	F	1	0.050	0.050	0.050	2.000

Conventional samples with multiple residues of up to 9 substances were stated (Fig.1). The use of prohibited chemical crop protection products in organic food production is common all over the world. This is confirmed by the results of official studies monitoring crop protection product residues in food of plant origin produced by organic methods, including studies in the USA, where 18% of samples contain residues (Benbrook, 2004), in the Czech Republic, 11% of samples (Report, 2008), Ireland – 10%, and Germany (7% of samples with residues). Gnusowski et al. (Gnusowski et al., 2011) states that in an official inspection of Polish organic fruits, vegetables, grains, and herbs (2004 - 2010, 957 samples), residues of crop protection products were found in 14.3% of raspberry samples and 6.3% of strawberry samples. The studies being the subject of this work indicate that residues were determined in 2.5% of raspberry samples and 17.4% of strawberry samples. In an official inspection of Polish conventional crops (2010 - 2011, 1350 samples) (Nowacka et al., 2011), residues of crop protection products were present in 81.5% of raspberry samples and 42% of strawberries, and MRL violations were noted for procymidone (3.4%) in strawberries. Out studies indicate, that residues were noted in 72.2% of raspberries and 50% of strawberries. The high percentage of samples with residues above MRL was mainly due to the presence of procymidone (5 samples) and dithiocarbamates (15 samples) in raspberry samples, for which MRL are 0.02 and 0.02 mg·kg^{-1}, respectively. In comparison, MRL for dithiocarbamates in a strawberry is equal to 10 mg·kg^{-1}, and for other detected fungicides, from 2 to 10 mg·kg^{-1}.

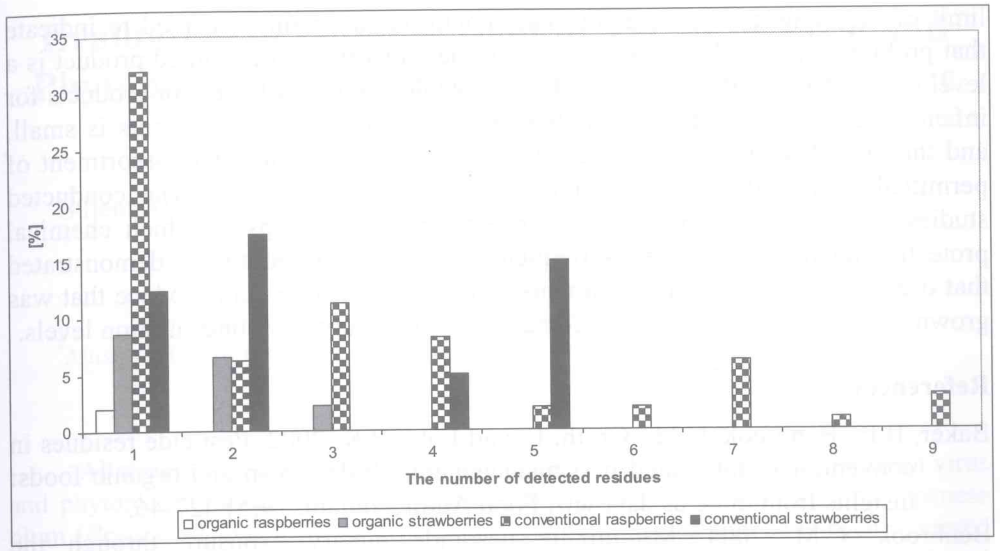

Fig. 1. Pesticide residues in ECO and conventional fruit samples

To compare average calculated concentrations relative to 1 sample, in ECO and conventional strawberries, they are equal to 0.03 mg·kg^{-1} each, and in the case of ECO and conventional raspberries, 0.03 mg·kg^{-1} and 0.05 mg·kg^{-1}, respectively. The highest average contents of residues relative to 1 sample were noted in conventional strawberries for DTC (0.35 mg·kg^{-1}), pyraclostrobin (0.17 mg·kg^{-1}), iprodione (0.14 mg·kg^{-1}), and in raspberries: fludioxonil 0.32 mg·kg^{-1}, cyprodinil 0,2 mg·kg^{-1}, for boscalid (0.134 mg·kg^{-1}), DTC (0.11 mg·kg^{-1}). To compare the proportion of average detected pesticide content in conventional strawberry samples to organic samples, it was assessed that it was the highest for the following substances: fenhexamid - 22.5, cyprodinil - 9.5, DTC - 4.9, pyraclostrobin - 3.6, boscalid - 1.6, biphentrin - 1.55 and chlorpyrifos - 1.4. A proportion of average content >1 means that organic products are less contaminated with this residue than food produced by a conventional system. These conclusions are also confirmed in other studies. Nonetheless, Polish conventional strawberries and raspberries contain residues at very low concentration levels. For instance, Baker et al. (Baker et al., 2002), have shown, that in the USA, organically grown foods consistently had about one-third as many residues as conventionally grown foods and about one-half as many residues as found in IPM/NDR samples. Conventionally grown and IPM/NDR samples were also far more likely to contain multiple pesticide residues than organically grown samples.

An organic product should not contain residues of crop protection products other than those permitted for use (Act, 2009). The highest acceptable levels of pesticide residues in organic crops are neither standardized in Poland nor in other countries of the EU, and some countries believe that there is no need to standardize them (Proposal, 2004). In general, residues should not be greater than the detection limit of the method. However, in many countries, the value accepted to indicate that prohibited pesticides were not used in the cultivation of a studied product is a level no greater than 0.01 mg kg^{-1} - the acceptable level established for products for infants is generally used. The number of farms using prohibited agents is small, and the fact of their use is most likely the result of an insufficient assortment of permitted means of crop protection (Commission Reg., 2008). The conducted studies show that strawberry and raspberry fruits are crops in which chemical protection against agrophages is frequently used. This research has demonstrated that organic fruit have smaller amounts of pesticide residue than produce that was grown conventionally, which also contain residue at very low concentration levels.

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Evaluation of Gummy Dent in Terms of Viral and Phytoplasma in Japanese Plum in Mersin Province of Turkey

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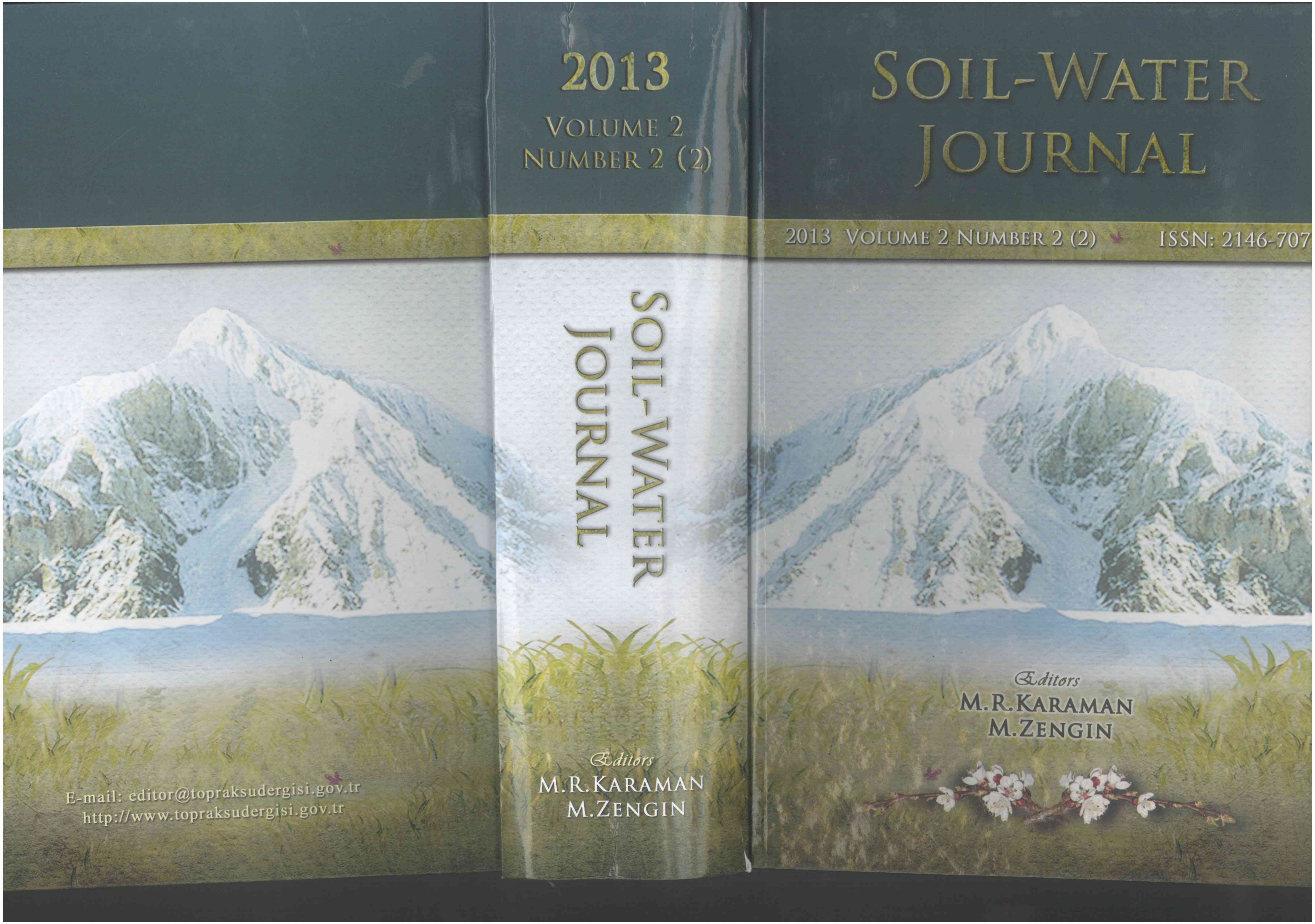
Abstract: This study was carried out to determine the relationship between virus and phytoplasma diseases in stone fruits which have gummy dent seen in Japanese plum (*Prunussalicina*) fruits in district of Mersin on 2012 which have been observed for 4-5 years. Leaf and shoot samples were analysed for virus with DAS-ELISA and RT-PCR for phytoplasma with Direct and Nested PCR. Fifty samples of 20 cm shoot with dent were taken from four district of Mersin province and tested for virus and phytoplasma diseases. Virus testing of Prunus dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Plum pox virus (PPV), Apple mosaic virus (ApMV) and Apple chloroticleafspot virus (ACLSV) were tested made with DAS-ELISA and moreover taking into account the negativeness of ELISA, RT-PCR also used for virus testing. No any viral pathogens were found. European stone fruit yellows phytoplasma (ESFY) was tested by direct and nested PCR and not found any of samples. All Japanese plum varieties in the region wereinvestigated in terms of gummy dent and it was seen only in Black Diamond and Black Beauty varieties. Because this problem, it can be concluded that ecologically sensitivity of these varieties and ecological situation has priority for gummy dent. Consequently, in determining of new species and varieties for the region, adaptation studies have to take account.

Keywords: European stone fruit yellows phytoplasma, Japanese plum, stone fruit virus

1. Introduction

The abundant ecological sources of Turkey bring to be origin of the many fruit species. Among those, plum which is one of the stone fruits has an important place with 268.696 tones plum production, in terms of both fresh and dried consumption, and industry (Anonymous, 2011). In the Eastern Mediterranean region, stone fruits have a huge potential in terms of early and fresh fruit consumption.Furthermore, depending on developments in communication at recent years consumer interests increased in new types of fruit. For this reason, in addition to the known and cultivated plum types, Japanese plum types which have attractive color, flavor, good firmness, large sized started to produce in different regions of the country and have very high chance to export. Turkey is one of the gene



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2013
VOLUME 2
NUMBER 2 (2)

SOIL-WATER
JOURNAL

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SOIL-WATER JOURNAL

2013 VOLUME 2 NUMBER 2 (2) ISSN: 2146-707

Editors
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