DETERMINATION OF PESTICIDE RESIDUES IN MOLEHIYA (*Corchorus olitorius*) GROWN IN NORTHERN CYPRUS BY USING QuEChERS METHOD

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCE OF NEAR EAST UNIVERSITY

By
AYŞE GEYLAN

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

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Approval of Director of Graduate School of Applied Science

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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Signature:
Date:

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ABSTRACT

Molehiya is one of the important minor crops of Turkish Republic of Northern Cyprus (TRNC) which is widely consumed by local people. The aim of this study was to determine pesticide residues and their levels in molehiya plants grown in TRNC. For this study original QuEChERS multi residue method which acetonitrile was applied for sample preparation, followed by LC-MS/MS and GS-MS/MS analysis of clean Molehiya extracts for 312 pesticides coming from different groups.

Molehiya samples were taken from twenty-two regions between June and September in 2015. As a results of analysis, 3.9 mg/kg (MRL: 0.7 mg/kg) cypermethrin, 1.8 mg/kg (MRL: 1.0 mg/kg) indoxacarb have been found in Bostancı molehiya samples and 0.06 mg/kg imidacloprid (MRL: 0.05 mg/kg) in Ozanköy molehiya samples.

Keywords: Molehiya; pesticide; residue; QuEChERS; North Cyprus

ÖZET

Molehiya, Kuzey Kıbrıs Türk Cumhuriyeti'nde (KKTC) yerel halk tarafından yaygın olarak tüketilen önemli minor ürünlerden biridir. Bu çalışmanın amacı KKTC'de yetiştirilen molehiya bitkilerindeki pestisit kalıntılarını ve seviyelerini belirlemektir. Bu çalışmada numune hazırlamada asetonitril uygulanan ve ardından temiz molehiya ekstraktlarının LC-MS/MS ve GS-MS/MS ile farklı gruplardan gelen 312 pestisitin analiz edildiği orijinal QuEChERS çoklu kalıntı metodu uygulanmıştır.

2015 yılı Haziran-Eylül aylarında 22 bölgeden molehiya numunesi alınmıştır. Yapılan analizler sonucunda Bostancı'da cypermethrin 3.9 mg/kg (MRL: 0.7 mg/kg), indoxacarb 1.8 mg/kg (MRL: 1.0 mg/kg) ve Ozanköy'de imidacloprid 0.06 mg/kg (MRL: 0.05 mg/kg) olarak bulunmuştur.

Anahtar Kelimeler: Molehiya; pestisit; kalıntı; QuEChERS; Kuzey Kıbrıs

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LIST OF ABBREVIATIONS

ADI:	Acceptable Daily Intake
AOAC:	Association of Official Agricultural Chemists
APCI:	Atmospheric Pressure Chemical Ionisation
CAC:	Codex Alimentarius Commission
CCPR:	Codex Committee on Pesticide Residues
CCRVDF:	Codex Committee on Residues of Veterinary Drugs in Foods
CI:	Chemical Ionisation
CRM:	Certified Reference Materials
DAD:	Diode Array Detector
EC:	European Commission
ECD:	Electron Capture Detector
EFSA:	European Food Safety Authority
EI:	Electron Impact
ESI:	Electrospray Ionisation
EU:	European Union
FAO:	Food and Agriculture Organization of the United Nations
FPD:	Flame Photometric Detector
GAP:	Good Agricultural Practices
GCB:	Graphitized Carbon Black
GC-MS/MS:	Gas Chromatography–Tandem Mass Spectrometry
GC-MS:	Gas Chromatography-Mass Spectrometry
IARC:	International Agency for Research on Cancer
IAEA:	International Atomic Energy Agency
IUPAC:	International Union of Pure and Applied Chemistry
JECFA:	Joint FAO/WHO Expert Committee on Food Additives
JMPR:	Joint FAO/WHO Meeting on Pesticide Residues

LC- MS	Liquid Chromatography–Mass Spectrometry
LC/MS-MS:	Liquid Chromatography–Tandem Mass Spectrometry
LOD:	Limit of Detection
LOQ:	Limit of Quantification
MeCN:	Acetonitrile
MeOH:	Methanol
MRLs:	Maximum Residue Limits
MRM:	Multi-Residue Method
NFA:	National Food Administration
NPD:	Nitrogen-Phosphorus Detector
OP:	Organophosphorus Pesticide
PFPD:	Pulsed Flame Photometric Detector
PSA:	Primary Secondary Amine
QC:	Quality Control
QuEChERS:	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD:	Relative Standard Deviation
SD:	Standard Deviation
SFE:	Supercritical Fluid Extraction
SIM:	Selected Ion Monitoring
SPE:	Solid-Phase Extraction
SRM:	Selected Reaction Monitoring
TRNC:	Turkish Republic of Northern Cyprus
WHO:	World Health Organization

CHAPTER 1

INTRODUCTION

Pesticides are one of the most important inputs in modern farming and will remain indispensable for the foreseeable future. Without agrochemicals it would be practically impossible to produce enough food to feed the world's growing population. As pesticides are hazardous substances, there is increasing concern about the safety and quality of food from such farms and in the surrounding environment.

As the use of pesticides has not decreased but increased, governments have introduced measures to restrict and regulate their use to protect the users of pesticides, consumers, and the environment.

Food control systems of countries' play a critical role in the regulation and educating farmers for the safe use of peticides. Pesticide registration is based on the assessment of very extensive research data on the toxicity and the fate of active ingredient, its metabolites and residue data obtained from supervised trials. The specified conditions of use in the registration document or use of pesticides according to *Good Agricultural Practices* (GAP) shall guide food safety stakeholders to ensure that foods are free from unsafe amounts of pesticides (Joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency Programme (IAEA), 2015).

Food control activities provide consumer protection that all foods for human consumption are safe and meet the quality requirements', and are accurately labelled as prescribed by law. Many countries have food control systems to achieve this goal. Food control systems may include food policies and legislation, food control management, diagnostic and analytical laboratories, food inspection, certification and enforcement, emergency preparedness and response, food-borne disease surveillance, and public information, education and communication. Achieving food safety is a shared responsibility contributed by different

types of stakeholders such as government, the food industry, consumers and their organizations, academic and scientific institutions.

Establishing a small team of representatives from the main government agencies responsible for food control such as food safety organisations, relevant units in agricultural/health ministries, food inspectorates, standards organizations, laboratories can provide practical support for information collection and analysis.

Responsibilities of government institutions, the food industry and consumers concerning food safety outcomes:

- The general level of food safety and quality in the country;
- The safety and quality of domestically produced food, imported and exported food;
- Public health outcomes, specifically the incidence and prevalence of food-borne diseases;
- The results and impact of food safety measures implemented by different stakeholders on daily basis and in response to emergencies; and
- The cost of food safety measures and regulations (Hopper & Boutrif, 2007).

1.1 Pesticides

Pesticides are chemicals which are widely used to control pests and pest-induced diseases, particularly in agriculture and human health (García-Garcíaet al., 2015). Pesticides can be classified according to their purpose of use as shown in Figure 1.1.

Extensive use of pesticides introduces a potential hazard to human and environmental health (García-García et al., 2015).

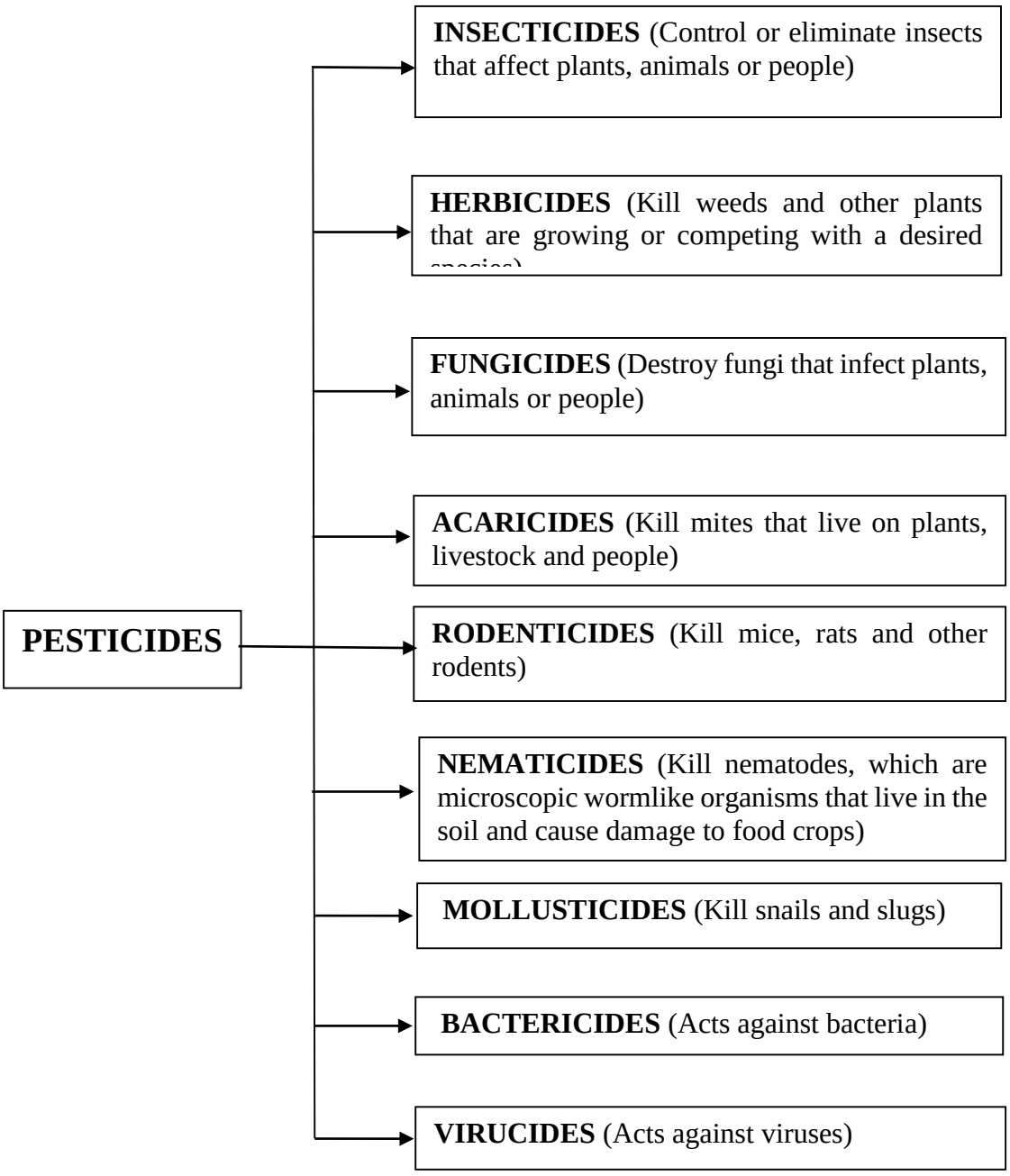

Figure 1.1: The classification of pesticides according to the type of pest they control (Toros and Maden, as cited in Yücel, 1997)

1.2 Effects of Pesticides on Environment

Pesticides effect the environment by point-source pollution and nonpoint-source pollution. Point-source pollution is the contamination originated from a specific and identifiable place; including pesticide spills, wash water from cleanup places, leaks from storage sites, and improper disposal of pesticides and their containers. Non-point source pollution is caused by contamination of a wide area including the drift of pesticides through the air, pesticide runoff into waterways, pesticide movement into ground water.

Environmentalists, agriculturalists and scientists are all aware of the long-term effects of pesticides as they contaminate streams and watercourses. Air may also be contaminated with pesticides because of application drift, post-application vapour loss and wind erosion of treated soil. Soil, vegetation and water bodies within the field margins may become contaminated due to wet and dry atmospheric deposition of pesticides and through surface runoff from pesticide-treated agricultural land (Cessna et al, 2005, as cited in Tiryaki & Temur, 2010)**.**

Fortunately most of the modern pesticides are organic and they are subject to biological decomposition. Via decomposition, pesticide compounds progressively break down to their component compounds, ions and elements, which in turn form simpler and generally less toxic compounds. Some decomposition products may incorporate into other organic substances via biological, chemical transformation mechanisms (Büyüksönmez et al., 1999).

Important processes, which determine the fate of a pesticide in soil, waters and the air, are:

a. Volatilization
b. Absorption by plants and animals
c. Sorption by organic and mineral matter
d. Transport in air, liquid or solid phases
e. Chemical and biological transformation and degradation.

All of these processes are influenced by pesticide properties (e.g. solubility, vapour pressure), soil and environmental conditions (e.g. temperature, moistureand soil pH), type of pesticide formulation and method of pesticide application (Kookanaa & Simpson, 2000).

1.3 Effects of Pesticides on Humans

Pesticides are chemicals to protect agricultural crops from biological hazards such as insect, weeds, fungi and other pests. In addition to their use in agriculture, pesticides are also used to protect human health from the vectors of tropical diseases, such as mosquitoes.

However pesticides are also potentially toxic to humans. They may induce adverse health effects on reproduction, immune or nervous systems and cancer. Before they can be authorized for use, pesticides should be tested for all possible health effects to assess any risk to humans.

Hazardous chemicals acoording to potential health effects can be classified as carcinogenic (to cause cancer), neurotoxic (to cause brain damage), or teratogenic (to cause damage to fetus). This classification process, called *hazard identification*, is the first step of *risk assessment*. An example of hazard identification is the classification of substances according to their carcinogenicity to humans carried out by the International Agency for Research on Cancer (IARC), the specialized cancer agency of World Health Organization (WHO).

The same chemical can have different effects at different doses, depending on the quantitiy of a person exposed to. It can also depend on the route by which the exposure occurs for example, ingestion, inhalation or injection (WHO, 2015).

Age, gender, socio-economic status, diet, health status, length of exposure and form, pesticide concentration are significant factors on the influence of a pesticide and on people under the influence of pesticides (Güler and Çobanoğlu, 1997, as cited in Oymen, 2014).

Classification of an agent as a carcinogenic hazard is an important indication that a certain level of exposure, for example from occupation, environment, food, etc., could result in an increased risk of cancer.

Risk assessment for pesticide residues in food, as conducted by the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues (JMPR), establishes a safe intake level. Acceptable Daily Intakes (ADIs) are used by governments and international risk managers, such as the Codex Alimentarius Commission (CAC), to establish maximum residue limits (MRLs) for pesticides in food. MRLs are enforced by national authorities to ensure that the amount of pesticide that consumers are exposed to in the food and they eat over a lifetime will not cause any adverse health effects.

IARC's hazard identification and the JMPR's risk assessment are complementary. For example, IARC may identify new evidence from scientific studies on the carcinogenicity of a chemical and, when necessary, JMPR conducts an evaluation or a re-evaluation of the safety of that chemical as it is used in food (WHO, 2015).

1.4 Maximum Residue Limits of Pesticides

Residues are the traces of pesticides left in treated products. A maximum residue level is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (GAP).

The amounts of residues found in food must be safe for consumers and must be as low as possible. The European Commission (EC) fixes MRLs for all food and feed. The MRLs for all crops and all pesticides can be found on the EC website / MRL Databox (EC, 2016).

Regulation (EC) No 396/2005 establishes the MRLs of pesticides permitted in products of plant or animal origin intended for human or animal consumption. MRLs are derived after a comprehensive assessment of the properties of the active substance and residue levels resulting from the GAP defined for the treated crops.

An essential precondition for setting MRLs is a risk assessment demonstrating consumer safety (consumer intake not exceeding the toxicological reference values).

The Regulation, fully applicable since September 2008, repeals the previous fragmentary legislation and replaces all national MRLs with harmonized European Union (EU) MRLs for all foodstuffs. The European Food Safety Authority's (EFSA) Pesticides Unit is responsible for the risk assessment of MRLs in accordance with the legislation. Every year, EFSA publishes an Annual Report on Pesticide Residues in the EU based on the monitoring information on pesticide residues in food. The EU MRL monitoring programmes are one of the most comprehensive food survey programmes worldwide, covering more than 60.000 food samples every year which are analysed for up to approximately 800 different pesticides (EFSA, 2015).

The FAO Panel on pesticide residues in food and the environment also evaluates pesticide residue data resulting from pesticide use according to GAP to estimate maximum residue levels in food and feed commodities. Under GAP, a pesticide is used for effective pest control, but leaves a residue that is the smallest amount practicable. The use must be safe for the user and the environment, and residues in food must be safe for the consumer.

MRLs are set by the CAC, acting as the risk manager. Draft MRLs for adoption by the CAC are elaborated by the relevant Codex committees, the Codex Committee on Pesticide Residues (CCPR) and the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), on the basis of scientific expert advice provided by the risk assessors- i.e. Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and Joint FAO/WHO Expert Committee on Food Additives (JECFA), respectively. The scientific advice developed by JMPR and JECFA aims to provide maximum residue levels for individual crops, plant and animal products, based on the results of scientific studies, so that these levels can be used by the relevant Codex committee to develop the draft MRLs, which may be adopted by the CAC (FAO & WHO, 2008).

There are national MRL lists about a maximum residue limits (MRLs) for every country in the world. MRL lists that are valid in international platform shall be prefered in order to avoid problems in international trade. Sometimes the combination of EU and FAO Codex limits can be harmonized and used (Tiryaki, 2011).

The MRL list of Turkish Republic of Northern Cyprus (TRNC) has been arranged to become in accordance with the EU's MRL list.

1.5 Pesticide Residue Analysis

Pesticide residue analysis plays crucial role in estimating the exposure of humans and the environment to pesticides, in controlling implementation of GAP in the field, in facilitating regulatory decisions and trading and in strengthening the consumers' trust towards food safety. In official government programmes and the private sector, end-point control is gaining importance world wide and there is a growing pressure on laboratories to improve cost-effectiveness and analytical performance, and to decrease sample run times. To address these needs, instrument manufacturers and residue analysts around the world are continuously developing and implementing new analytical techniques and approaches with the aim of simplifying and speeding-up procedures, improving quality and the scope of analysis and reducing chemical consumption and manual labour (Anastassiades & Scherbaum, 2004).

Achieving ultimate goal in fair practice in international trade is based among other factors, on the reliability of analytical results. Particularly in pesticide residue analysis, depended not only on the availability of reliable analytical methods, but also on the experience of the analyst and on the implementation of "good laboratory practices" (CAC/GL, 2003).

In 2003, Anastassiades et al. introduced a new method of analysis to determine pesticide residues, which they called the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. The method utilizes acetonitrile (MeCN) for extraction (1:1 mL MeCN: g sample) using vortex mixing followed by addition of 4:1 (w/w) anhydrous MgSO4: NaCl (0.5 g salts per g sample) to induce partitioning of the MeCN extract from the water in

the sample. After centrifugation, 1 mL of the extract is then mixed with 25 mg primary secondary amine (PSA) sorbent + 150 mg anhydrous MgSO4 in a simple approach that is termed dispersive solid-phase extraction (dispersive-SPE) cleanup. The extract is centrifuged again and transferred to an autosampler vial for analysis by gas chromatography/mass spectrometry (GC/MS) and/or other technique (Lehotay et al., 2004).

The QuEChERS method has several advantages over traditional methods of pesticide residue analysis, as follows:

a. High recoveries higher than 85% are obtained for a wide polarity and volatility range of pesticides, including difficult analytes like pyrethroids, dichlorvos, methamidophos, thiabendazole, omethoate andimazalil;
b. Very accurate and precise results are achieved because an internal standard is used to correct for commodity to commodity water content differences and volume fluctations;
c. Solvent consumption and waste is very small, and no chlorinated solvents are used;
d. A single person can perform the method without much training or technical skill;
e. High sample throughput: 10-20 pre-weighed samples in 30-40 min;
f. Only a single re-usable piece of *glassware* (a 50 mL Teflon centrifuge tube) is used per sample which is very easy to clean;
g. Despite the speed and ease of the method, the method is still quite rugged because extract cleanup is done to remove fatty acids and other organic acids which are ubiquitous in foods;
h. Very little bench space is needed thus the method can be carried outin a small laboratory;
i. Acetonitrile is added by dispenser to an unbreakable vessel that is immediately sealed, thus solvent exposure to the worker is minimal (no chlorinated solvent is used);
j. The method is cost-effective in a comparison of other methods; and
k. The only devices needed are a chopper, balance and centrifuge (no blender, SPE manifold or evaporation apparatus) to carry out the sample preparation method.

The combination of liquid chromatography–tandem mass spectrometry (LC/MS-MS) with GC/MS can currently provide the most effective and efficient means to both quantify and identify hundreds of pesticide analytes in a variety of matrices (Lehotay et al., 2004).

1.6 Agriculture in TRNC

Agriculture is one of the backbone sectors of TRNC in production, exportation and as national revenue. Cyprus is a semiarid island having inadequate and irregular rainfall; naturally water is crucial input for agriculture. Inadequate underground sources are only water supply in the island.

Out of 329,890 hectares total area in TRNC, 56.71 percent which is 187,068 hectares is suitable for agriculture. Climatic conditions, shortage of water sources & their usage and the existence and convenience of agricultural lands are main restrictive factors concerning agriculture.

Irrigated lands are mainly used for citrus, deciduous fruits, grapes and some varieties of vegetables cultivation. The rest of the lands has been using for traditional cereal production. In total agricultural production, share of cereals is 70 percent; legumes 15; vegetables, fruits and others 15%. Most of the exporting products are irrigated agricultural crops (Agricultural Structure and Production, 2010).

Cyprus has an extremely rich flora in terms of genetic diversity and is localized on two genetic diversity centers of many cultivated plants. There are many domestic varieties grown for centuries in Cyprus but they are under the risk of extinction due to the pressure of modern agriculture, especially use of hybrid cultivars (Yilmaz et al., 2012).

Jute (*Corchorus olitorius*) is one of the irrigated minor crops among vegetables which is widely called as “molehiya” in TRNC. Jute is also known as Long-fruited jute, Tossa jute, Jute mallow, Jew’s mallow, Bush okra and West African sorrel. It is called Moroheiya in Japan and Saluyot in the Philippines (İlhan et al., 2007).

1.7 Molehiya: Jute (*Corchorus olitorius*)

Corchorus (Malvaceae), a genus of about 40-100 species of flowering plants, is distributed in the tropics of both hemispheres. In Northern Cyprus, this genus is represented with two annual species, namely *Corchorus olitorius L*. and *C. trilocularis L*. Molehiya is a native plant of tropical Africa and Asia and has been spread to Australia, South America, and some parts of Europe (Meikle, 1977, as cited in İşeri et al., 2013). Besides having industrial importance in world jute production, it has agricultural importance as a widely cultivated and consumed crop in Cyprus, and in some Arabic countries as *Molukhyia*. In Arabic countries, it is also used for the treatment of fever, chronic cystitis, aches and pains, dysentery, enteritis, and pectoral pains (Zakaria et al., 2006, as cited in İşeri et al., 2013).

The crop is a good source of vitamins A and C, fiber, minerals including calcium and iron and other micronutrients. Molehiya is widely consumed as a *healthy vegetable* in Japan, as it contains abundant carotenoids, vitamin B1, B2, C and E, and minerals (Matsufuji et al., 2001, as cited in İlhan et al., 2007).

Molehiya contains high levels of all essential amino acids except methionine which is at marginal concentrations. It has high protein levels and is, along with other leafy species, the main source of dietary protein in many tropical countries (Tulio et al., 2002, as cited in İlhan et al., 2007).

The seeds are used as a purgative and leaves as demulscent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria. On preliminary analysis, seeds have been found to contain cardenolide glycosides (Gupta et al., 2003, as cited from İlhan et al., 2007). The methanol (MeOH) extracts of Molehiya seeds have shown a broad spectrum of antibacterial activity (Pal et al., 2006, as cited in İlhan et al., 2007).

Molehiya is an annual herb with slender stems. Molehiya is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asia in such countries as the Philippines and Malaysia, as well as in tropical Africa, Japan, South America, the Caribbean and Cyprus. In West African countries particularly Ghana, Nigeria and Sierra Leone, where staple diets consist of starchy food-stuffs such as rice, cassava,

maize and yams, leafy vegetables are used to complement such staple foods (Tulio et al., 2002 as cited inİlhan et al., 2007).

It is cultivated to provide bark for the production of fibres (Jute) and its mucilaginous leaves are used as a vegetable (Abou Zeid AHS, 2002; Meikle, 1977, as cited in İlhan et al., 2007).

Molehiya is one of the traditional dishes in Northern Cyprus originated from Arab Cuisine (Eşiyok et al., 2010).

1.7.1 Morphologic properties of molehiya

Some of the morphologic properties of molehiya (Figure 1.7) are as following:

a) It look like hemp with yellow flowers
b) It has sesame sized seeds
c) Its colour turned from green to black when it is dry
d) Depending on culvitation and care conditions the height of the plant change from 50 to 200 cm and it can reach up to 400 cm.
e) Boughs located on plant stem as spiral shaped and side branches formed by those boughs (Eşiyok et al., 2010).

Figure 1.7: Pictures of molehiya in the field

1.7.2 Climate and soil conditions for molehiya cultivation

Molehiya is cultivated in the places where tropic and subtropic climate prevail. Besides this, it can be cultivated better in mild climate where almost nofrost does occur. Although the optimum soil structure for plant growth is alluvial, the plant can also be cultivated in clay soil and heavy soil under irrigation. Neutral soils that have pH levels between 6.5 and 7.5 are being preferred. The optimum temperature for plant's growth is 27-32°C. The plant is drought resistant. However, under convenient moisture conditions plants can show optimum growth and its leaves' quality and yield increase.

Relative humidity of 70%-80% is favorable for successful cultivationin the case that its leaves are consumed as food. The insufficient moisture conditions have negative impact on the quality of the plant and make leaves hard and fibrous. The plantation time in Cyprus is in the middle of April.

1.7.3 Harvest

Molehiya can reach to harvest maturity 40-50 days after sowing under good agricultural practices (Eşiyok et al., 2010).

The 4.43 percent of total molehiya production of TRNC was used as vegetables in 2009. According to 2009 Agricultural Statistics, molehiya was cultivated in 316 decares of total irrigated lands which is equal to 0.34 percent of 93453 decares (TRNC The Ministry Agriculture and Natural Resources, Statistics and Planning Division, 2010). Distribution of molehiya cultivated areas and molehiya production in TRNC were summarized in Table 1.7a and Table 1.7b.

Table 1.7a: The distribution of molehiya cultivation area in irrigated lands of TRNC (Agricultural Structure and Production, 2010)

Districts	Molehiya Cultivation Area (Decares)
Lefkoşa	147
Gazimağusa	71
Girne	1
Güzelyurt	94
İskele	3
TRNC (Total)	316

Table 1.7b: 2009 molehiya production by regions in TRNC (Agricultural Structure and Production, 2010).

Districts	Molehiya Area, decare (da)	Production, tone (t)
TRNC	**316**	**330**
LEFKOŞA	**147**	**140**
Lefkoşa (Center)	-	-
Değirmenlik	147	140
Ercan	-	-
GAZİ MAĞUSA	**71**	**66**
G. Mağusa A	33	28
G. Mağusa B	-	-
Akdoğan	8	7
Geçitkale	24	26
Gönendere	5	5
GİRNE	**1**	**2**
GirneDoğu	-	-
GirneBatı	1	2
Boğaz	-	-
Çamlıbel	-	-
GÜZELYURT	**94**	**119**
Güzelyurt	44	54
Lefke	49	65
İSKELE	**3**	**3**
İskele	-	-
Mehmetçik	3	3
YeniErenköy	-	-

1.8 The Use and Control of Pesticides in TRNC

In TRNC, pesticides are licensed as EU compatible by a Joint Committee of four participants from Ministry of Agriculture, Natural Sources and Food and three participants from Ministry of Health. Pesticides cannot be used in the country without permission of Pesticides Control Committee. Among these Governmental Organizations, Department of Agriculture-Ministry of Agriculture, Natural Sources and Food is also responsible to determine pesticide residues in food products both produced in TRNC and imported from abroad. By end-point control, consumption of food products which contain pesticide residues more than acceptable limits can be prohibited. Such products are destructed by the authorization of laws and regulations of Pesticides Control Committee. The Department of Agriculture not only controls food commodities but also prepares and pursues pest control programmes in TRNC to ensure safe use of pesticides and to provide more healthy products to consumers (Tarladan Sofraya Yediklerimiz, 2014).

During the period of January till October 2015, samples were taken from 973 fields to determine pesticide residues for compliance with MRLs. Residues in 26 samples out of 973 were reportedly found to be above MRLs and therefore they were destructed. Uncompliant crop rate in domestic products was 2.68 percent (Sebze ve Meyve Sorunsuz, 2015), whereas EU average rate was 3.5 percent (Sebze ve Meyve Sorunsuz, 2015; Tarladan Sofraya Yediklerimiz, 2014).

In Table 1.8 common insects and diseases and pesticides used against these pest on green leafy vegetables grown in TRNC were summarized. As can be seen in the table, various insecticides and also fungicides have been usingto control insects and fungi on vegetables.

The aim of this study was to determine pesticide residues in molehiya that is one of the most important minor crops of TRNC by using QuEChERS multi residue method.

Table 1.8: Common insects and diseases and pesticides used against these pest on green leafy vegetables (Tarım Dairesi, 2014)

The Name of Diseases	**Active Ingredients of Pesticides**
Gray mold diseases in vegetables (*Botriyts cinerea*)	Captan %50
Septoria leaf spot –vegetables (*Septoria apiicola, Septoria lycopersici*)	Bordeaux mixture
Lettuce downy mildew (*Bremia Lactucae*)	Captan %50 Ametoctradin 300g/l+Dimethomorph 225g/l
Collapse disease in vegetable seedling (*Phythium spp., Rhizoctonia spp., Fusarium spp., Alternaria spp., Sclerotinia spp.*)	Copperoxychloride %50 Maneb %80 Captan %50 Thiram %80
Spinach downy mildew (Pero nospora farlinosa)	Ametoctradin 300g/l + Dimethomorph 225g/l
Whiteflyin vegetables Tobacco whitefly (*Bemisia tabaci*) Greenhouse whitefly (*Trialeurodes vaporariorum*)	Chlorpyrifos ethyl, 480g/l Cypermethrin, 200g/l Cypermethrin, 250g/l Deltamethrin, 25g/l LambsaCyhalothrin + Buprofezin, 20+100 g/l Pirimiphos-methyl, 500 g/l Yellow sticky traps (570-580nm)
Thrips in vegetables Tobacco thrips (*Thripstabaci*) Flower thrips (*Frankliniella occidentalis*)	Pirimicarb;%50
Aphids Cotton aphid (*Aphis gossypii*) Black bean aphid (*Aphis fabae*) Green peach aphid (*Myzuspersicae*) Potato aphid (*Macrosiphum euphorbiae*)	Deltamethrin, 25g/l Pirimicarb;%50 Pirimiphos-methyl,500g/l Thiamethoxam,240 g/l Malathion, 190 g/l Malathion, 500 g/l Clothianidin %50

CHAPTER 2

THEORETICAL FRAMEWORK

2.1 Sampling and Sample Processing

A good residue analysis starts with taking representative samples from fields or orchards or from a consignment. For the analysis of pesticide residues both composite samples and primary samples (e.g. animal tissues, plant portions for metabolism studies) are taken. Since the distribution of pesticide residues is not homogenous in the natural units and there is a great variation in their average residue content, the samples must be properly taken, reduced in size, packed, labelled, and protected against contamination, decomposition and alteration (Ambrus, n.d; Kateman & Buydens, 1993; Snedecor & Cochran, 1980; Youden, 1967).

The way of sampling should be performed when controlling the MRL conformity of commodities is prescribed in several national and international guidelines that define the minimum number of units and sample amount that should be sampled (Anastassiades & Scherbaum, 2004).

The whole sample material should be thoroughly minced and mixed to obtain an analytical portion representing the average residue content of the sample (Ambrus, n.d; Kateman & Buydens, 1993; Snedecor & Cochran, 1980; Youden, 1967).

2.2 Contamination

Samples should be very well packaged and sealed in polythene or nylon bags and transported and processed separately to avoid contamination (SANCO, 2009). Samples should not be stored near chemicals. Containers, that may have been used for the storage of agricultural chemicals, should not be used for packaging or transport.

2.3 Laboratory Sample

Laboratory sample is the sample sent to, or received by, the laboratory. A representative quantity of material is removed from the composite sample. The laboratory sample can be the whole or a part of the composite sample. Units should not be cut or broken to produce the laboratory sample(s) and replicate laboratory samples should be prepared (CAC/GL, 1999).

Samples must be identified clearly and indelibly, in a way that prevents inadvertent loss or confusion of labelling. The use of marker pens containing organic solvents should be avoided for labelling bags containing samples to be analysed for fumigant residues, especially if an electron capture detector (ECD) is to be used.

Samples must be transported under appropriate conditions to the laboratory in clean containers and robust packaging. Rapid transportation to the laboratory, preferably within one day, is essential for samples of fresh products. The condition of samples delivered to the laboratory should approximate to that acceptable to purchaser, otherwise samples should normally be considered unfit for analysis (SANCO, 2009).

Extensively spoiled or degraded laboratory samples should not be analysed. When possible, laboratory samples should be prepared immediately after arrival and in any event, before any significant physical or chemical changes have taken place. If a laboratory sample cannot be prepared without delay, it should be stored under appropriate conditions to keep it fresh and to avoid deterioration. Generally, laboratory samples should not be stored longer than 3 days before preparation. Dried or similarly processed samples should be analysed within their stated shelf life (European Standard, 2007).

2.4 Sample Preparation

Sample preparation begins with sample processing and ends with the generation of the final extract used for instrumental analysis. Before the analytical portion is taken (sub-sampling), intensive cutting, chopping and blending or grinding is necessary to reduce particle sizes and ensure statistically well-mixed homogenates that can be used for checking the compliance of the entire laboratory sample with MRLs. A thorough comminution reduces the variability

of results within replicate test portions and improves the accessibility and extractability of residues (Anastassiades & Scherbaum, 2004).

On receipt, each laboratory sample must be allocated a unique reference code by the laboratory.

Sample processing and sub-sampling to obtain analytical portions should take place within the shortest time practicable before visible deterioration occurs.

Sample processing must be in accordance with the definition of the commodity and the part(s) to be analysed (SANCO, 2009).

The whole laboratory sample (in most cases 1-2 kg) needs to be comminuted. From each test sample, any parts that would cause difficulties with the homogenisation process should be removed. In the case of stone fruits, the stones shall be removed. A record of the plant-parts that have been removed shall be kept. Precautions should be taken to avoid any losses of juice or flesh. This is called as test sample. Calculation of the residue shall be based on the mass of the original test sample including the stones (European Standard, 2007).

Leafy vegetables (except group 4/ brassica leafy vegetables) are foods derived from the leaves of a wide variety of edible plants including leafy parts of Group 1 vegetables. The entire leaf may be consumed. According to related Codex Guideline, whole commodity is analysed after removal of obviously decomposed or withered leaves (Codex Alimentarius, 2000).

Where the homogeneity of the test sample is not sufficient or the extraction of residues may be significantly compromised due to large particle sizes, intensive comminution should be performed using appropriate means (European Standard, 2007).

2.5 Sample Portion (Analytical Portion)

Individual test portions are taken from the comminuted test sample and analysed immediately.

2.6 Interference

Interference from natural constituents of samples is frequent especially in the case of food commodities. Analyte peak enchancement can be observed when matrix components, or other pesticides in a complex mixture, compete with analytes for active sites on the injector parts and protect susceptible pesticides from adsorption or decomposition. Consequently, inaccurate false positive results can be obtained if calibration solutions in pure solvent are used.

Another problem, opposite to that described above, is the "matrix-induced" response diminishing effect. Polar active sites originated from non-volatile matrix components that have accumulated in the inlet or in the front section of a capillary column from matrix deposit, were found to be responsible for adsorption and decomposition of analytes in the injection port. Though the matrix enhancement effect was reported more often than the diminishing effect, the latter might cause even more serious problems during analysis since it leads to wrongly reported low values.

The most common approach to eliminate systematic errors arising from matrix induced chromatographic response enhancement is to use matrix matched solution for calibration (Soboleva et al., 2000).

If the interference takes the form of a response overlapping that of the analyte, a different clean-up or determination system may be required. If it is not practicable to eliminate interference, or to compensate for it by matrix-matched calibration, the overall accuracy (bias) and precision of analysis should nonetheless comply with acceptable criteria (SANCO, 2009).

2.7 QuEChERS Sample Preparation

The approach is known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for multiclass, multiresidue analysis of pesticides in a variety of matrices. As the name implies, the QuEChERS sample preparation approach has many practical advantages over traditional methods. The limit of quantitation (LOQ) of the method was designed to be

less than 10 ng/g for all analytes, and the linear dynamic range should permit analysis beyond 10,000 ng/g depending on the analyte and instrumentation.

In terms of analytical scope, nearly all pesticides except those relatively few that contain carboxylic acid groups can be monitored by the QuEChERS approach.

Dispersive-SPE with PSA effectively removes many polar matrix components common in food matrices, such as organic acids and certain polar pigments. PSA sorbent retains some pesticides containing carboxylic acid groups, such as daminozide and 2,4-D, chlorothalonil, dicofol, folpet, captan, captafol, dichlofluanid, and tolylfluanid tend to degrade in MeCN as pH increases and in the presence of light, thus results for those pesticides are more variable depending on the matrix and conditions used. The additional use of C_{18} sorbent in dispersive-SPE can provide additional cleanup of lipids. If no pesticides with planar structures (*e.g.* thiabendazole, terbufos, quintozene, hexachlorobenzene) are included among the analytes, then graphitized carbon black (GCB) can also be used in dispersive-SPE to provide additional clean-up of sterols, chlorophyll, and structurally planar matrix components.

For samples having water content below 80 %, cold water (< 4 °C) shall be added leading to total water content in the tube of approximately 10 g (Table 2.7) (Manual for IAEA by AGES, n.d.).

Table 2.7: Typical water content and the amount of water to be added to the corresponding test portions for some food commodities

Sample Type	Mass, g	Water, g	Remarks
Fruit/vegetables water content > 80 %	10	0	
Fruit/vegetables water content 25–80 %	10	X	x = 10 g – watercontent in 10 g sample
Cereals	5	10	soak for max. 5 min
Dried fruits	5	10	Optional: add 850 gcold water to 500 gfrozen dried fruit and homogenize
Leaves	5	10	soak for max. 5 min
Spices, herbs (fresh)	2	10	soak for max. 5 min
Spices, herbs (dried)	2	0	

2.8 Chromatographic Separation and Determination

LC/MS-MS or GC/MS plays a key role and provide the most effective and efficient means to both quantify and identify hundreds of pesticide analytes in a variety of matrices in one run.

Various MS detection systems can be used, such as single or triple quadrupole, ion trap, time of flight, orbitrap. Typical ionisation techniques are: electron impact (EI), chemical ionisation (CI), atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). Different acquisition modes may be used such as full-scan, selected ion monitoring (SIM), selected reaction monitoring (SRM) and multiple reaction monitoring.

Nowadays, selective detectors for GC (ECD, flame photometric detector (FPD), pulsed flame photometric detector (PFPD), nitrogen-phosphorus detector (NPD) and LC (diode array detector (DAD), fluorescence) are less widely used as they offer only limited specificity. Their use combination with different polarity columns does not provide unambiguous identification. These limitations may be acceptable for frequently found pesticides, especially if some results are also confirmed using a more specific detection

technique. Such limitations in the degree of identification should be acknowledged when reporting the results (SANCO, 2013).

The measurement may be performed using various instruments, instrument parameters and columns. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities (European Standard, 2007).

2.9 Calibration for Quantification and Matrix-Matched Standards

The sample extracts and calibration standard solutions are injected into the GC or LC instruments in appropriate sequences. This may involve bracketing of the sample extracts with the calibration solutions.

Matrix-matched standards are prepared in the same way as assolvent-based standards. However, extracts of blank samples are used instead of pure MeCN. To minimize errors caused by matrix induced effects during chromatographic analysis, it is the best to choose similar commodities (e.g. apple for apple samples, carrot for carrot samples, etc.) (European Standard, 2007).

2.10 On-Going Performance Verification (Routine Recovery Determination)

The over-all aim of QC (Quality Control) is to ensure that analytical results are of adequate accuracy for their intended application. If the analytical system or systems available are found to be incapable of supplying data of adequate accuracy, the case for performing analysis at all should be examined. The way in which QC can be implemented depends greatly on the nature of the work of the laboratory concerned.

There are several distinct categories. For instance, an active laboratory might:

a) analyse numerous large batches of similar materials,
b) analyse large batches of samples of widely differing matrix or determinand concentration and
c) perform a wide variety of different tests in small batches.

Each situation will require a different approach to QC and strategy of implementation (Bura, n.d.).

Where practicable, recovery of analytes of interest should be measured with each batch of analyses. If this requires a disproportionately large number of recovery determinations, the minimum acceptable frequency of recovery may be as given in Table 2.10 adobted from SANCO, 2009. The choice must include at least 10 % of the representative analytes per detection system. However, the number of representative analytes in each batch must not be less than 5 per detection system. Analysis of reference materials is a preferable option to use, though rarely practical due to the lack of certified reference materials (CRMs) providing that the materials contain the relevant analytes at appropriate levels (SANCO, 2009).

Table 2.10: Frequency for routine recovery check (performance verification)

	Representative analytes	**All other analytes**
Minimum frequency of recovery	10% of representativeanalytes (at least 5 perdetection system) in each batch of analyses	Within a rolling programme to include all other analytes at least every 12 months, but preferably every 6 months
	Within a rolling program covering all representative analytes as well as different types of commodities, at least at the level corresponding to the reporting limit.	At least at the level corresponding to the reporting limit.

CHAPTER 3

RELATED RESEARCH

In recent years, there are various researches about pesticide residues in fruits and vegetables in literature. Some of researches are summarised below:

In Marchis et al.'s study QuEChERS was modified and applied in the analysis of 9 organophosphate and 1 pyrethroid pesticides in raw materials for animal feeding introducing an additional liquid liquid partition step. They concluded that additional step allowed samples to be concentrated avoiding any solvent evaporation prior to the instrumental analysis. Once the method was optimized, it was carried out a pesticide- quantitation study using a GC-MS SIM multi-residue analysis. 45 samples of maize and soybean coming from Northern Italy (Piedmont Region) were analysed. 30 samples out of 45 organophosphate pesticides were containing Chlorpyrifos up to 12.4 mg/kg, while no Deltamethrin was detected (Marchis et al., 2012).

QuEChERS the most recent method has already been widely accepted by the international community of pesticide residue analysts and a lot of publications already deal with this method in its original form or variations of it (Lehotay et al. 2010). The QuEChERS method effectively covers a very wide analyte scope, including highly polar pesticides as well as highly acidic and basic ones. Additional advantages of the method were reported as high sample throughput and low amounts of solvent, glassware and bench space requirements. Analytical methodologies must be capable of residue measurement at very low levels and also provide unambiguous evidence to confirm both the identity and quantitiy of any residue detected (Sungur & Tunur, 2012).

Not only determination of pesticide residues but also other agrochemicals in food matrices is a great challenge mainly because of the small quantities of analytes and large amounts of interfering substances which can be co-extracted with analytes and, in most cases, adversely affect the results of an analysis. However, safety concerns require that agrochemicals of the

wide range of chemical properties (including acidic, basic and neutral) should be monitored. Because of the wide variety of food matrices, the sample must initially be cleaned up before final analysis. That is why the analytical chemist is faced with the need to invent new methodologies for determining such residues to be determined in a single analytical run. To accomplish the goal, QuEChERS methodology has been developed. So far, it has been achieved promising results by liquid or gas chromatographic analysis, including pesticides, but also acrylamide, pharmaceuticals and veterinary drugs (Wilkowska & Biziuk, 2011).

In a study, the sample preparation methods for multiresidue analysis of pesticides in vegetables were investigated by using cucumber and leek. Results show that the optimum sample preparation conditions for normal vegetables were as follows: The ratio of acetonitrile to water was 3:1 during C18 solid-phase extraction. The pH values prior to homogenization and liquid extraction were 7 and 5, respectively. The matrix interference was complex with Chinese chive. Adding small amount of phosphoric acid before homogenization could effectively diminish matrix interference, which leads to the recovery of 70% to 120% for more than 90 percent of 76 different types of pesticides (Shujuan, 2011).

Golge and Kabak (2015) modified and then validated QuEChERS method for the determination of 109 selected pesticides in tomatoes. The recovery ranged from 77.1% to 113.2%, with repeatabilities of 4.4–19.2% and within-laboratory reproducibilities of 7.1–18.4%. The limit of detection values (LODs) for target analytes in tomato extract were between 0.5 and 10.8 μg/ kg, and the LOQs were between 1.3 and 30.4 μg/kg. The expanded measurement uncertainty was not higher than 30% for all target analytes. Residues of acetamiprid, azoxystrobin and triadimefon were identified and measured in 9.6% of tomato samples, ranging from 0.015 to 0.37 mg/kg.

Nowadays, liquid chromatography coupled with mass spectrometry (LC-MS) is becoming one of the most powerful techniques for residue analysis of polar, ionic or low volatile pesticides in fruits and vegetables (Sungur & Tunur, 2012).

A method based on LC-MS/MS was developed for sensitive determination of a number of less GC-amenable organophosphorus pesticides in cabbage and grapes. For extraction,

several solvents were evaluated with respect to the possibility of direct injection, matrix-induced suppression or enhancement of response and extraction efficiency. Ethyl acetate was the most favourable solvent for extraction, although a solvent switch was required before injection. Extracts were analysed on a C18 column with polar endcapping. The final method is straightforward and involves extraction with ethyl acetate and a solvent switch to 0.1% acetic acid/water without further cleanup. Recoveries were between 80 and 101% with RSD less than 11% (n=5). The LOQ was 0.01 mg/kg and limits of detection were between 0.001 and 0.004 mg/kg (Mol et al., 2003).

A new multi-residue method for determination of pesticide residues in wide variety of fruit and vegetables, using the National Food Administration (NFA) ethyl acetate extraction and determination by means of LC-MS/MS was presented. The method has been validated for 57 different pesticides and metabolites. Representative species from different commodity groups were chosen as matrices in order to study the influence of different matrices on recoveries. The fortification levels were 0.01-0.5 mg/kg. Matrix effects were tested for all matrices by means of standard addition to blank extracts. The matrix effect, expressed as signal in solvent compared to signal in matrix, was found to be small. The obtained recoveries were with a few exceptions, in the range 70-100%. The proposed method is quick and straightforward and no additional clean-up steps are needed. The method can be used for the analysis of all 57 pesticides in one single determination step at 0.01 mg/kg (Jansson et al., 2004).

Jicheng et al. (2011) investigated the applicability of supercritical fluid extraction (SFE) in organophosphorus pesticide analysis in vegetables. Fortification experiment and orthogonal test based on the trichlorfon as investigating target were conducted to optimize the extraction procedure in SFE by varying the CO_2 flow, extraction oven temperature, outlet temperature and pressure. The best efficiency was achieved at 18 mL/min, 60°C, 80°C and 350 bar, respectively. The analytical screening was performed by capillary gas chromatography equipped with FPD. Recoveries for the majority of pesticides from spiked samples with the fortification level of 0.04-0.10 mg/kg ranged from 80.5 to 97.3%, with relative standard deviations (RSD) 2.9 to 8.4. The detection limits were from 0.008 to 0.035 mg/kg for FPD, and meeting the requirement set of native standard. The optimized SFE method is a simple,

low cost, environment-friendly and an effective preparation method for determination of pesticide multi-residue at trace level in leafy vegetables in comparison with solid-liquid extraction method.

In recent years high residual amounts of pesticides, mainly parathion and methamidophos, have been found in vegetable samples through routine monitoring efforts conducted by the Ministry of Agriculture of China. Hundreds of cases of acute poisoning resulting from the contamination of agricultural products with pesticides are reported every year. In this study, it was examined pesticide residue levels in vegetables (cucumber, celery, tomato, green pepper and eggplant) from four sources in Shenyang City. Results show that organophosphorus pesticide (OP) levels in cucumber, celery and tomato samples were above the safe limits for consumption. Generally, samples from the larger supermarkets of Shenyang were safer than those from retailers and from the farmers' market. Parathion was the most commonly detected residue. Omethoate, phorate and methyl parathion, currently prohibited due to their highly toxic nature, were also detected in some samples (Li et al., 2011).

QuEChERS method for determination of thiophanatemethyl, carbendazim, metalaxyl, fluazifop-P-butyl, chlorpyrifos and lambda-cyhalothrin in five brassica vegetables by LC-MS/MS and GC-ECD has been developed. The average recoveries of six pesticides in five brassica vegetables were in the range of 77.4%– 117.4% with RSD of 3.7–10.8%. Totally 48 open field trials on five brassica vegetables were conducted at two locations in two different seasons. The residue dynamics and final residues of the six pesticides at three preharvest intervals in different vegetables were compared. All six pesticides had the longest half-lives in cabbage (2.1–3.5 days). Residues of carbendazim (sum of thiophanate-methyl and carbendazim), metalaxyl, chlorpyrifos and lambda-cyhalothrin had similar trend in different brassica vegetables. The maximal concentrations of these pesticide residues were found in kale (0.28–10.9 mg/kg). Fluazifop-P-butyl residues were at low levels in all five brassica vegetables (<0.01–0.03 mg/kg). Cabbage, red cabbage, Brussels sprouts and kohlrabi had no significant difference in all six pesticide residues and could be classified in a subgroup of Head Brassicas. Cabbage should be selected as the representative commodity.

Considering the highest residues in kale and its different morphology, kale should not be classified into the subgroup of Head Brassicas (Yu et al., 2015).

The aim of the present study was to investigate the presence of 175 pesticides in various vegetables and fruit samples grown in different regions of Hatay. In cucumber samples, acetamiprid residues were found more than the maximum acceptable level according to Turkish Food Codex and EU MRLs. In other samples, the detected residue amounts were less than maximum residue limits listed in the Turkish Food Codex and EU MRLs (Sungur & Tunur, 2012).

In pesticide residue analysis, analyte concentrations are generally too low and samples too complex to be analysed without preliminary sample preparation. The main goal of sample preparation is therefore to provide a sample fraction, which is enriched in all analytes of interest and as free as possible from interfering matrix components that will certainly be present in the extract. Any analyte losses occurring in this step cannot be compensated for in the subsequent measurement steps. Thus, sample preparation is a crucial part of whole analytical process.

Excellent recoveries and low variations have been achieved in intralaboratory validation experiments of QuEChERS Method (Anastassiades & Scherbaum, 2004).

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Sampling of molehiya

Laboratory samples were taken in accordance with Directives of Department of Agriculture-Ministry of Agriculture, Natural Sources and Food between June and September in 2015.

As shown in the Figure 4.1a and Figure 4.1b, individual portions for the laboratory sample were taken from different points of the field. Two bags of laboratory sample were prepared from each field and immediately transfered to the Government Laboratory for the analysis.

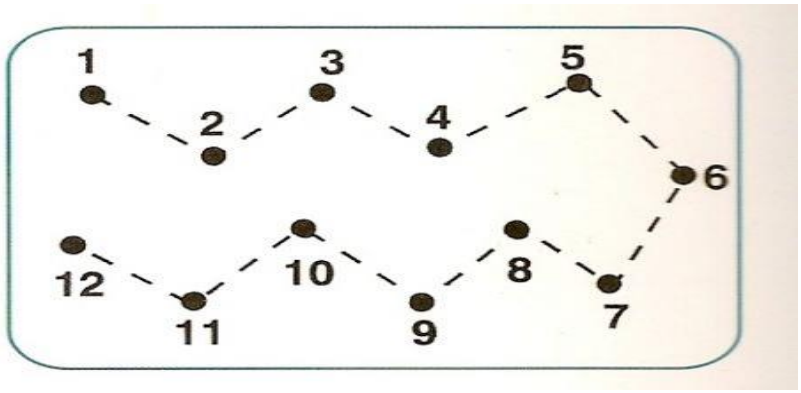

Figure 4.1a: The way of how samples were taken from different points of the field

Figure 4.1b: Pictures from sampling of molehiya in the field

Laboratory sample bags containing approximately half kilograms of molehiya were sealed and labeled properly. The label contains following informations related to sample:

Seal number, sample code, type of sample, place of sampling, date and time of sampling, farmers' name and address and amount of sample (Figure 4.1c).

Figure 4.1c: Sealing and labeling of laboratory sample bag of molehiya after sampling

Twenty-two samples have been taken from thirteen different regions namely Bostancı, Gaziveren, Yuvacık, Akçay, Ozanköy, Yeni Boğaziçi, Gazimağusa, Yıldırım, Haspolat, Aydınköy, Demirhan, Taşpınar and Çatalköy between June and September in 2015.

Name of villages in TRNC where samples in 2015 summer were taken according to the programme of Department of Agricultures were given in Table 4.1 and Figure 4.1d. In the Table 4.1, numbers given to villages represent different regions of same village.

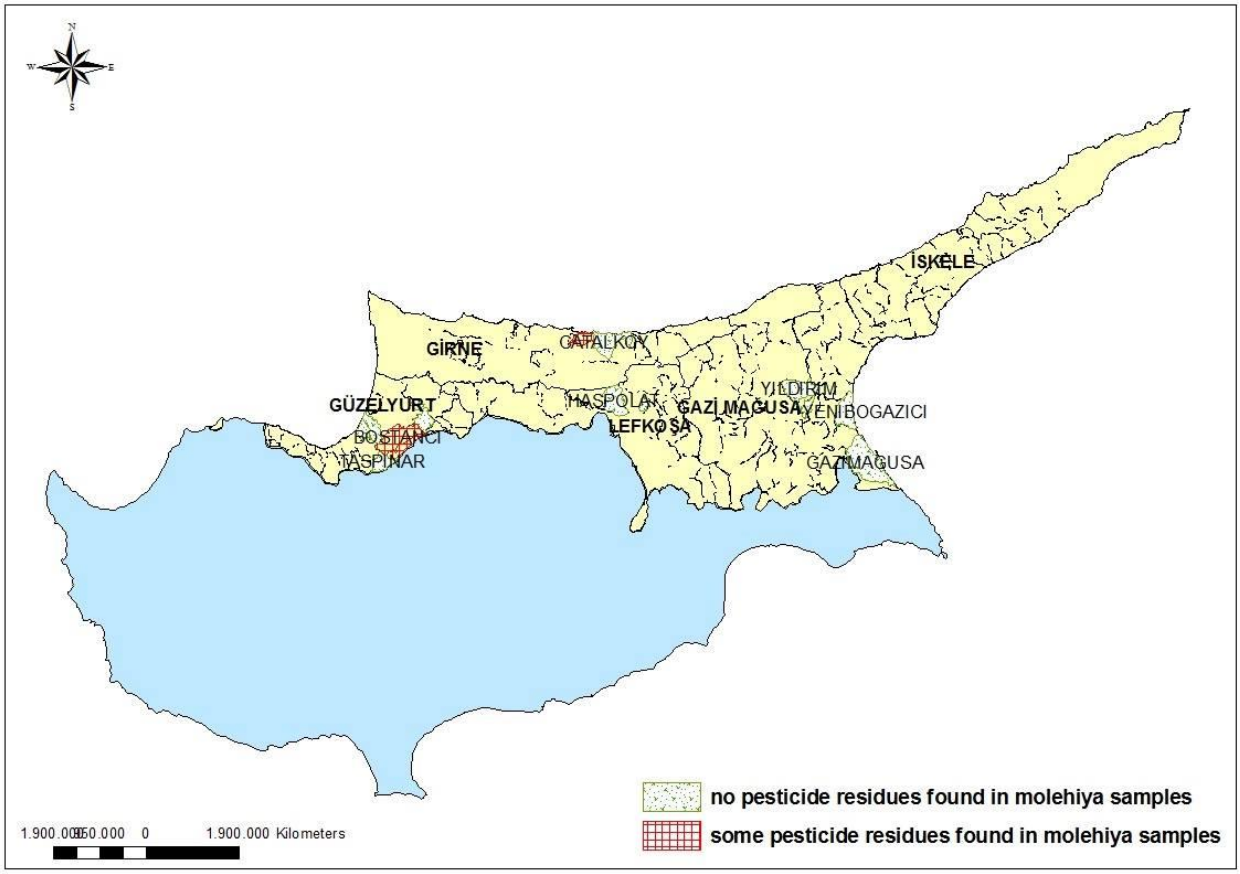

Figure 4.1d: Regions in TRNC where Molehiya samples were collected in 2015

Table 4.1: The districts and regions where samples were taken for the analysis

Districts	Regions
Gazimağusa	Yeni Boğaziçi-1
	Yeni Boğaziçi-2
	Gazimağusa
	Yıldırım
Güzelyurt	Akçay-1
	Akçay-2
	Aydınköy-1
	Aydınköy-2
	Bostancı-1
	Bostancı-2
	Bostancı-3
	Gaziveren
	Yuvacık
	Taşpınar
Lefkoşa	Haspolat-1
	Haspolat-2
	Haspolat-3
	Demirhan-1
	Demirhan-2
Girne	Ozanköy
	Çatalköy

Before harvest, presampling is carried out according to the Governmental Plant Protection Programme. If any sample at harvest time contains any pesticide residue more than MRL, sampling is repeated one week later. In the meantime any harvest from the field is not allowed. For checking compliance with MRL, sampling can be repeated maximum three times before harvest. If samples from these fields contain still uncompliant high residues, these crops are destroyed by Department of Agriculture.

4.2 Method

4.2.1 Sample processing and preparation

a. Molehiya leaves belonging to laboratory sample were taken into chopper's bowl and comminuted 30 sec- 1 min as can be seen in Figure 4.2a.

Figure 4.2a: The preparation of molehiya leaves for analysis

b. Sub samples (analytical portions) were taken right away for extraction and clean-up.
c. Some 7.5 g of well-homogenized molehiya sample was weighted in to 50 mL centrifuge tube and 7.5 g of water was added.

Figure 4.2b: The preparation of well-homogenized molehiya leaves for analysis

d. 15 mL of MeCN containing 1 % of acetic acid was added on the sample and shaken for 1 min on a multi shaker.

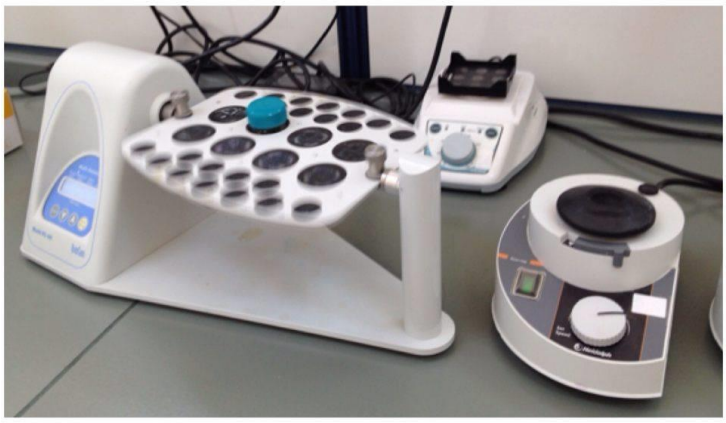

Figure 4.2c: Shaking of the extraction tubes with acetonitrile

e. 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate were added and shaken for 1 minute by using multi shaker**.**

In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. After the addition of the salt mixture into the centrifuge tube, tubes should be immediately shaken vigorously for few seconds. The 1 min extraction of the entire batch may be performed in parallel after the salts have been added to all the samples.

f. Centrifuge the tube for 5 min at 4100 rpm.

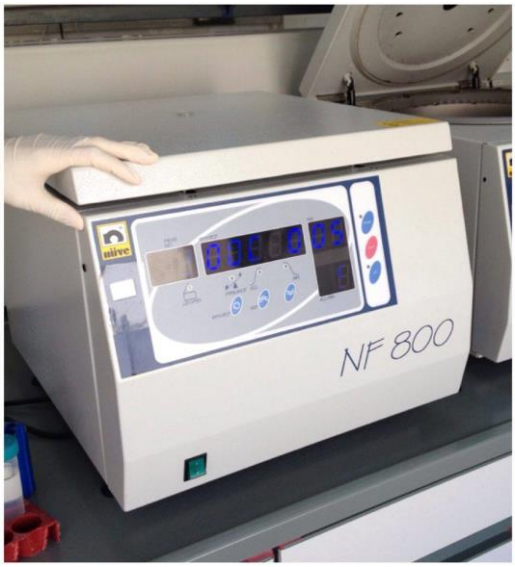

Figure 4.2d: Centrifugation process

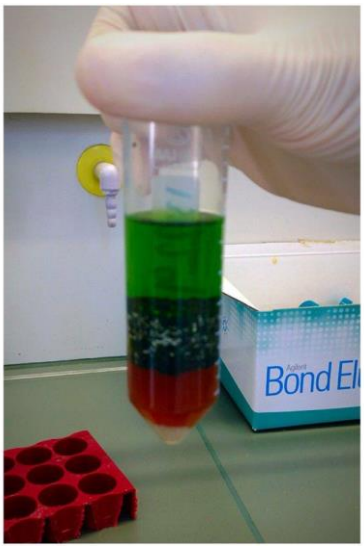

Figure 4.2e: Phase separation after extraction process

4.2.2 Clean-up with amino-sorbent (Dispersive SPE with PSA)

a. An aliquot of 8 ml of the MeCN phase from second extraction step was transferred into a polypropylene -single use centrifuge tube already containing 0.2 g PSA and 1.2 g of magnesium sulfate (Quechers kit for fruits & vegetables). For 1 ml of extract 25 mg PSA and 150 mg magnesium sulfate are necessary.
b. Close the tube and shake vigorously for 1 min and centrifuge (for 5 min at >4100 g).
c. Aliquot is filtered by using 0.45µ filter then transferred to vials.
d. Vials are injected to LC-MS/MS and Gas Chromatography–Tandem Mass Spectrometry (GC-MS/MS).

Flow diagram of the QuEChERS method was summarized in below Figure 4.2f.

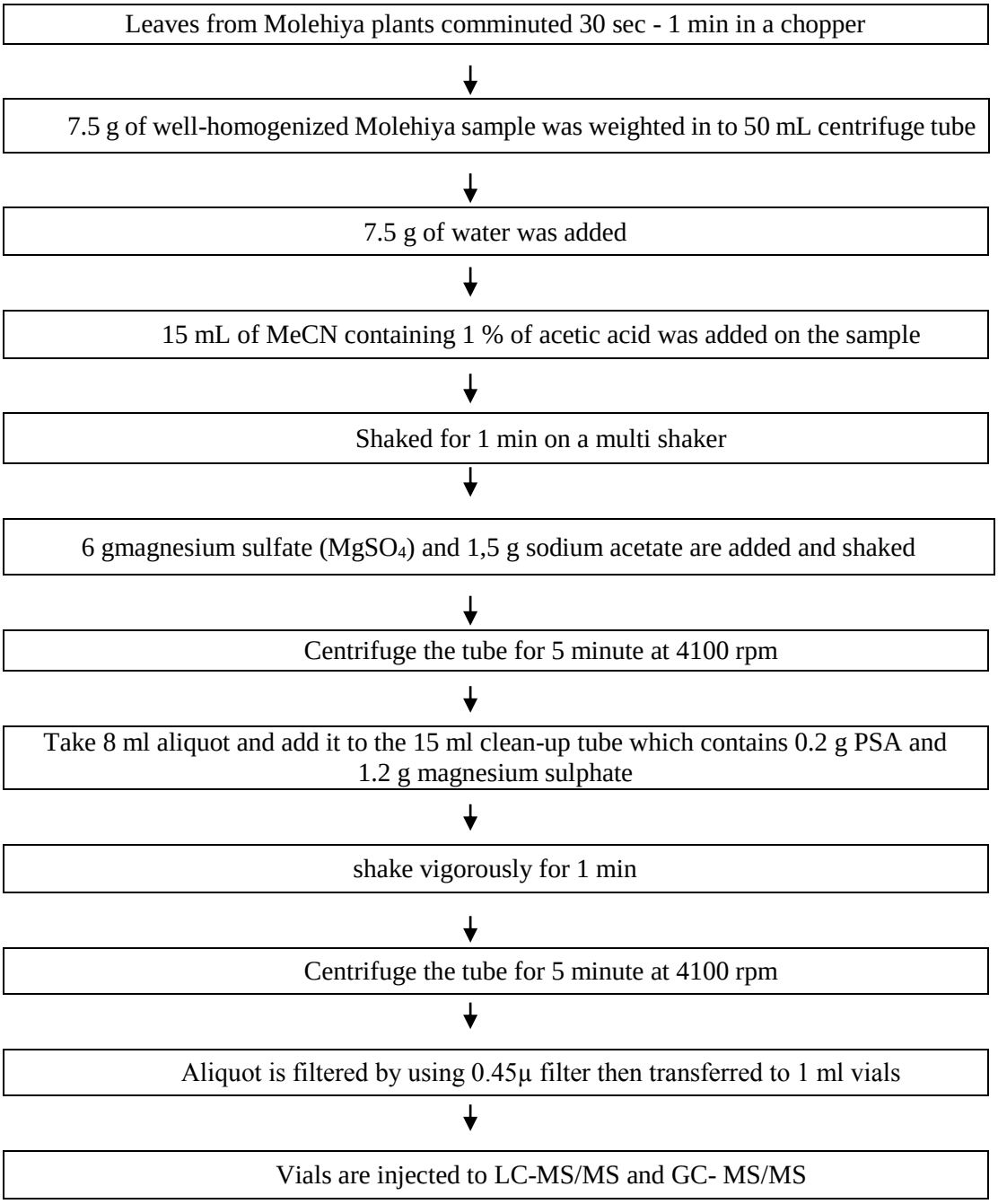

Figure 4.2f: Flow-chart of QuEChERS method

4.2.3 Standard solutions

1000 mg/kg stock solutions of every single pesticide standards are prepared in acetonitrile. Secondly, an intermediate standard mixture of 10 mg/kg is prepared by using stock solutions in acetonitrile. After that, this mixtureis used to prepare 0.010, 0.025, 0.050, 0.100 mg/kg solutions by diluting with acetonitrile. Once each concentration of standard solutions is injected to LC-MS/MS instrument by using the method prepared for residue analysis, retention time, peak height and area for standard concentrations have been saved and calibration curve was prepared.

Table 4.2: Name of the pesticides analysed by using LC-MS/MS and GC-MS/MS

Pesticides **Method of Analysis: LC-MS/MS**	
1-Abamectine	138-Haloxyfob – 2 – ethoxyethyl
2-Acephate	139-Hebtenophos
3-Acetamiprid	140-Hexaconazole
4-Aclonifen	141-Hexythiazox
5-Alachlor	142-Imazalil
6-Aldicarb	143-Imazamox
7-Aldicarb-Sulfoxid	144-Imibenconazole
8-Allethrin	145-Imidacloprid
9-Ametryn	146-Indoxacarb
10-Aminocarb	147-Iodosulfuron
11-Amitraz	148-Ioxynil
12-Atrazine	149-Iprodione
13-Azamethiphos	150-Iprovalicarb
14-Azinphos- Methyl	151-Isofenphos
15-Azoxystrobin	152-Isoprocarb
16-Benalaxyl	153-Isoproturon
17-Bendiocarb	154-Kresoxim – Methyl
18-Benfuracarb	155-Lambda – cyhalothrin
19-Bensulfuron-	156-Lenacil
20-Bentazone	157-Linuron
21-Benzoximate	158-Lufenuron
22-Bifenthrine	159-Malaoxon
23-Bitertanol	160-Malathion
24-Boscalid	161-Mandipropamid
25-Bromuconazole	162-Mecarbam
26-Bupirimate	163-Mepanipyrim
27-Buprofezin	164-Metaflumizone
28-Butocarboxim	165-Metalaxyl

29-Butylate	166-Metamitron
30-Cadusafor	167-Metazachlor
31-Carbaryl	168-Metconazole
32-Carbendazim	169-Methabenzthiazuron
33-Carbofuran	170-Methamidophos
34-Carbosulfan	171-Methidathion
35-Carboxin	172-Methiocarb
36-Chinomethionate	173-Methomyl
37-Chorantranilirole	174-Methoxyfenozide
38-Chlorfenvinphos	175-Metobromuron
39-Chlorfluazuron	176-Metolachlor
40-Chloridazon	177-Metosulam
41-Chloroxuron	178-Metoxuron
42-Chlorpropham	179-Metrafenone
43-Chlorpyrifos – Methyl	180-Metribuzin
44-Chlorpyrifos	181-Metsulfuron – methyl
45-Chlorthiophos	182-Mevinphos
46-Cinidon – ethyl	183-Monocrotophos
47-Cinosulfuron	184-Monolinuron
48-Clethodim	185-Monuron
49-Clodinafob	186-Myclobutanil
50-Clofentezine	187-Naled
51-Clomazone	188-Napropamide
52-Clothianidin	189-Neburon
53-Coumaphos	190-Nicosulfuron
54-Cyanazine	191-Nitenpyram
55-Cyazofamid	192-Norfluazuron
56-Cyclanilide	193-Nuarimol
57-Cycloate	194-Ofurace
58-Cyflufenamid	195-Omethoate
59-Cymoxanil	196-Oxadiazon
60-Cypermethrin	197-Oxadixyl
61-Cyproconazole	198-Oxamyl
62-Cyprodinil	199-Paclobutrazol
63-Cpromazine	200-Penconazole
64-Dazomet	201-Pendimethalin
65-Deltamethrin	202-Phenmedipham
66-Desmedipham	203-Phenthoate
67-Desmethyl pirimicarb	204-Phosalone
68-Diafenthiuron	205-Phosmet
69-Diazinon	206-Phosphamidone
70-Dichlofluanid	207-Piperonyl butoxide
71-Dichlorfos	208-Pirimicarb
72-Diclobutrazol	209-Pirimiphos – ethyl
73-Diclofob – Methyl	210-Pirimiphos – Methyl
74-Dicrotophos	211-Pirimisulfuron
75-Diethofencar	212-Prochloraz
76-Difenoconazole	213-Profenofos
77-Diflufenican	214-Promecarb
78-Dimethachlor	215-Prometryn

79-Dimethenamid	216-Propachlor
80-Dimethoate	217-Propamocarb Hcl
81-Dimethomorph	218-Propaquizafob
82-Diniconazole	219-Propargite
83-Dioxathion	220-Propetamphos
84-Diphenamid	221-Propham
85-Diuron	222-Propiconazole
86-Dodemorph	223-Propoxur
87-Dodine	224-Propoxycarbazone
88-Edifenphos	225-Propyzamide
89-Emamectine Benzoate	226-Prosulfuron
90-Endosulfan sulfate	227-Prothiophos
91-Epoxiconazole	228-Pymetrozine
92-EPTC	229-Pyraclostrobin
93-Ethiofencarb	230-Pyraflufen – ethyl
94-Ethiofencarb – Sulfon	231-Pyrazophos
95-Ethiofencarb – Sulfoxid	232-Pyridaben
96-Ethion	233-Pyridaphenthion
97-Ethirimol	234-Pyridate
98-Ethofumesate	235-Pyrimethanil
99-Ethoprophos	236-Pyrimidifen
100-Etofenprox	237-Pyriproxyfen
101-Etoxzazole	238-Quinalphos
102-Famoxadone	239-Quinoxyfen
103-Fenamidone	240-Quizalofob – p –ethyl
104-Fenamiphos	241-Resmethrin
105-Fenarimol	242-Simazine
106-Fenazaquin	243-Spinosad
107-Fenbuconazole	244-Spiroxamine
108-Fenhexamid	245-Sulfentrazone
109-Fenoxaprop – p – ethyl	246-Tau – fluvalinate
110-Fenoxycarb	247-Tebuconazole
111-Fenpiclonil	248-Tebufenpyrad
112-Fenpropathrin	249-Tebutam
113-Fenpropidin	250-Teflubenzuron
114-Fenpropimorph	251-Tepraloxydim
115-Fenpyroximate	252-Terbumeton
116-Fenthion	253-Terbuthylazine
117-Fipronil	254-Terbutryn
118-Flamprob-M	255-Tetraconazole
119-Florasulam	256-Tetramethrin
120-Fluazifob – p – butyl	257-Thiabendazole
121-Fludioxonil	258-Thiacloprid
122-Flufenacet	259-Thiamethoxam
123-Flufenoxuron	260-Thiophanate – methyl
124-Fluometuron	261-Tralkoxydim
125-Fluquinconazole	262-Triadimefon
126-Fluridone	263-Triadimenol
127-Flurochloridone	264-Tri – allate
128-Flusilazole	265-Triasulfuron

129-Flutolanil	266-Triazophos
130-Flutriafol	267-Tribenuron – methyl
131-Fonofos	268-Trichlorfon
132-Foramsulfuron	269-Trifloxystrobin
133-Formothion	270-Triflumizole
134-Fosthiazate	271-Triflumuron
135-Fuberidazole	272-Triflusulfuron
136-Furathiocarb	273-Triforine
137-Halosulfuron – methyl	274-Zoxamide
Pesticides **Method of Analysis: GC-MS/MS**	
275-(3) – hydroxycarbofuran	295-Endrin
276-Acetochlor	296-297 Fenvalerate
277-Aldrin	298-Fenchlorphos
278-BHC, alpha -	299-Fenitrothion
279-BHC, beta -	300-Fensulfothion
280-BHC, gamma – (Lindane)	301-Hexachlorbenzene
281-Bifenazate	302-Folpet
282-Bromopropylate	303-Oxyfluorfen
283-Butachlor	304-Parathion
284-Chlorothalonil	305-Phorate
285-Cyfluthrin	306-Procymidone
286-289 DDT	307-Quintozene
290-Dicofol	308-Tetradifon
291-Dieldrin	309-Tolclofos – Methyl
292-Diphenylamine	310-Tolyfluanid
293-Endosulfan, alpha -	311-Trifluralin
294-Endosulfan, beta -	312-Vinclozolin

4.2.4 Calibration

For the calibration of LC-MS/MS and GC-MS/MS instruments, 4 point – matrix matched calibration standards were used before chromatographic run of the samples. The lowest calibration level was 0.010 mg/kg. The new calibration curve is prepared everyday for quantification.

4.3 Chemicals and Reagents

Dispersive SPE 15 ml, Fruits and Vegetables, Association of Official Agricultural Chemists (AOAC)

QuEChERSExtract Tubes, AOAC Method

Ammonium Formate	Fluca, puriss, p.a. for mass spectroscopy, ≥99.0%
MeCN	Merck, Lichrosolv
Acetic Acid	Merck, glacial acetic acid
MeOH	Merck, Lichrosolv
0.45μm Polypropylene Cellulose Filter	Minisart RC 25

4.4 Equipment and Instruments

Analytical Balance	Boeco; 0.01 g; max:4100 g
Precision Scales	Sartorius Dual Range; 0.01 mg; max:220g
Multi shaker	*Biosan Multi Rotator-Multi RS-60*
Centrifuge	Nüve, NF800
Shredder	Boğaziçi- MPS 10.01
Automatic pipette	Eppendorf, 10-100 μl; 100-1000 μl; 0.5-5 ml
LC-MS/MS	Schimadzu-AB-MDS Sciex
LC-MS/MS	*Schimadzu 8040*
GC-MS/MS	*Schimadzu 8030*

4.5 Chromatographic Conditions

4.5.1 LC-MS/MS conditions

SCHIMADZU-8040 LC-MS/MS Instrument Parameters:

Flow:	0.5 ml/min
Injection volume:	20 μl
Column:	Inertsil ODS-4 (3 μm, 2.1x 50mm)
Run Time:	12 min
Mobile Phase:	A: 5 mM Ammonium format in water
	B: 5 mM Amonium format in MeOH

Table 4.5a: LC-MS/MS Gradient Programme

Time	Module	Events	Parameters
6.5	Pumps	Pump B Conc.	95
7.5	Pumps	Pump B Conc.	95
8	Pumps	Pump B Conc.	5
12	System Controller		Stop

Pumps:

Pump A Model:	LC-20ADXR
Pump B Model:	LC-20ADXR
Pumping Mode:	Binary Flow
Total Flow:	0.4000 ml/min
Pump B Conc:	5.0%
B Curve:	0
Pressure Range (PumpA/B):	10-330 Bars

Autosampler:

Model:	SIL-20AC
Rinsing Volume:	500 µl
Needle Stroke:	52 mm
Rinsing Speed:	35 µl/sec
Sampling Speed:	15.0 µl/sec
Purge Time:	25.0 min
Rinse Dip Time:	0 sec
Rinse Mode:	Before and after
Control Vial Needle Stroke:	52 mm

System Controller:

Model:	CEM-20A Lite
Sample	
Event 1:	Off
Event 2:	Off

4.5.2 Mass spectrometer method parameters

The mass spectrometry method properties were as follows:

CID Gas:	Use the data in the tuning file
Conversion Dynode:	Use the data in the tuning file
Acquisition Mode:	MRM
Polarity:	Positive/Negative
Dwell Time:	1

4.5.3 GC-MS/MS conditions

SCHIMADZU-8030 GC-MS/MS Instrument Parameters:

Column oven Temp.:	90 °C
Injection Temp.:	260°C
Injection Mode:	Splitless
Sampling Time:	1.00 min
Carrier Gas:	He
Prim. Pres:	300-500
Row Control Mode:	Pressure
Pressure:	120.0 kPa
Total Flow:	89.0 mL/min
Column Flow:	1.69 mL/min
Linear Velocity:	48.1 cm/sec
Purge Flow:	3.0 mL/min
Split Ratio:	50.0

Table 4.5b: GC-MS/MS Oven Programme

Program	**Column Oven Temperature**		
	Rate	**Final Temperature (°C)**	**Hold Time**
0	-	90.0	2.00
1	30.00	150.0	0.00
2	10.00	200.0	0.00
3	15.00	300.0	7.00

Total Program Time:	22.67 min
Column	
Name:	Restek RXI-5SILMS
Thickness:	0.25 µm
Length:	30.0 m
Diameter:	0.25 mm

4.6 Recovery Check: Acceptability of Analytical Performance for Routine Recoveries

When the mean recovery is calculated from one commodity group, acceptable limits should normally be within the range of the mean recovery +/- 2x RSD. Results may be corrected using within laboratory reproducibility (routine on going recovery) data or repeatability. However, a range of 60-140 % is used in routine pesticide residue analysis. Recoveries outside the above mentioned range require re-analysis of the samples but may be acceptable in some specific cases. Where the individual recovery is unacceptably high and no residues are detected, it is not necessary to re-analyse the samples to prove the absence of residues. However, consistently high recovery should be investigated. If a significant trend occurs in recovery, or potentially unacceptable (RSD beyond ± 20 %) results are obtained, analytical chemist should respond to an out-of control and investigate (SANCO, 2009).

Control molehiya samples were fortified with cypermethrin, deltamethrin, imidacloprid, indoxacarb and chlorpyrifos at the level of 0.025, 0.0255, 0.0249, 0.025 and 0.0255 mg/kg respectively and analysed. Recovery check has been especially carried out with cypermethrin, deltamethrin, imidacloprid, indoxacarb and chlorpyrifos, since they were found in molehiya samples collected from field in 2015. Average recovery, standard deviation (SD) of recoveries and RSD were calculated by using MS Excel and following formula:

$$\%\boldsymbol{RSD} = \left(\frac{\boldsymbol{SD}}{\textbf{Mean Rec.}}\right) * \mathbf{100} \qquad (4.1)$$

Then RSD value is used as below to give the best estimate of the residue content in a sample and certify compliance of a commodity.

$$\boldsymbol{R_{max-min} = R_i \pm (k * RSD * R_i)} \quad (4.2)$$

$R_{max-min}$:	Maximum and minimum residue amount (mg/kg)
R_i:	Residue amount measured in the sample (mg/kg)
k:	Expansion factor which is 2 by International Union of Pure and Applied Chemistry (IUPAC) at the level of 95% confidence
RSD:	Repeatability
RSD *Ri:	Combined standart uncertainty of the analysis
k* RSD* Ri:	Expanded Uncertainy of the analysis

Recoveries of the pesticides appeared in the range of 81- 108%. Overall recovery, SD and RSD values were found as 91.26%, 11.24 and 12.32% respectively. The method is considered valid, since all individual recoveries, overall recovery and RSD were within the specified acceptance criteria (70% ≤ Q ≤ 120% and RSD ≤ 20%) for 5 compounds in molehiya (Table 4.6).

Table 4.6: Results of recovery check

	% Recovery 1	**% Recovery 2**	**% Recovery Avarage**	**Mean Recovery**	**SD**	**% RSD**
Chlorpyrifos	85.7	83.4	84.6	**91.26**	11.24	**12.32**
Cypermethrin	96.0	101.6	98.8			
Deltamethrin	85.3	81.4	83.4			
Imidacloprid	80.0	82.8	81.4			
Indoxacarb	110.4	106.0	108.2			

CHAPTER 5

RESULTS AND DISCUSSION

Molehiya samples were taken from different regions of TRNC between June and September in 2015. Two molehiya samples out of 22 samples were containing pesticide residues. The others can be considered compliant, as their residue levels are below detection limits or MRLs. As can be seen in Table 5a, Ozanköy samples were containing delthamethrin at the level of 0.15mg/kg; 0.059 mg/kg of imidacloprid and 0.071 mg/kg of indoxacarb residues. Cypermethrinand indoxacarb residues were detected in Bostancı-3 molehiya samples at the level of 3.9 mg/kg and 1.8 mg/kg respectively.

Table 5a: Pesticide residues and their residue levels in 2015 molehiya samples

REGIONS	PESTICIDES	RESIDUE LEVEL (mg/kg)	MRL[a] (mg/kg)
OZANKÖY	Deltamethrin	0.15	0.5
	İmidacloprid	**0.059**	0.05
	Indoxacarb	0.071	1.0
BOSTANCI-3	Cypermethrin	**3.9**	0.7
	Indoxacarb	**1.8**	1.0

[a]MRLs evaluated according to EU limits revised on 09/06/2005

Interpretation of a residue value and the decision on the compliance of a sample representing a field with the MRL may be quite different depending on whether the uncertainty of the results is taken into account or not. Residue levels found in the samples were evaluated by taking into account recovery percent and repeatability of the results and estimated minimum and maximum residue content of the samples (Table 5b).

Table 5b: Pesticides and their residue levels, Rmin, Rmax and MRLs in 2015

Year	Pesticides	Residue Level (mg/kg)	Rmin	Rmax	MRL (mg/kg)
2015	Deltamethrin-O[a]	0.15	0.113	0.187	0.5
	İmidacloprid-O[a]	**0.06**	0.045	**0.075**	0.05
	Indoxacarb-O[a]	0.07	0.053	0.087	1.0
	Indoxacarb-B[b]	**1.80**	**1.357**	**2.243**	1.0
	Cypermethrin-B [b]	**3.90**	**2.940**	**4.860**	0.7

[a]Ozanköy samples
[b]Bostancı samples

According to that evaluation, indoxacarb and cypermethrin levels of Bostancı molehiya samples can be considered uncompliant with MRL values of these pesticides, as **Rmin** and **Rmax** values are higher than MRL. However imidachloprid level of Ozanköy molehiya samples can be considered compliant, as Rmin value is lower than MRL.

When we traced back 2014 residue data for molehiya, 0.019 mg/kg deltamethrin, 0.036 mg/kg chlorpyrifos in Serhatköy samples; 0.079 mg/kg cypermethrin in Bostancı samples; 2.5 mg/kg azoxystrobin, 0.062 mg/kg buprofezin, 0.077 mg/kg deltamethrin in Yuvacık sample; 1.1 mg/kg azoxystrobin in second sampling of Yuvacık samples and 0.02 mg/kg deltamethrin in Gaziveren samples had been detected (Table 5c). From these results, only Yuvacık molehiya samples' buprofezin level was higher than MRL. Molehiya samples from other regions found compliant at the second sampling, then Yuvacık found compliant at the third sampling. In 2015, 3.9 mg/kg cypermethrin was detected in Bostancı molehiya samples, whereas they were containing only 0.079 mg/kg cypermethrin in 2014.

Although pesticide residues in molehiya samples grown in 2014 were found in more regions, residue levels in 2014 were lower than those in 2015. In 2015, residues were found only in two regions; however, the residue levels are higher than MRLs.

Table 5c: Pesticides and their residue levels in 2014

REGIONS	PESTICIDES	RESIDUE LEVEL (mg/kg)	Rmin	Rmax	MRL[a] (mg/kg)
SERHATKÖY	Deltamethrin	0.019	0.015	0.025	0.5
	Chlorpyrifos	0.036	0.03	0.05	0.05
BOSTANCI[b]	Cypermethrin	0.079	0.06	0.1	0.7
YUVACIK[b]	Azoxystrobin	2.5	1.885	3.116	15.0
	Buprofezin	**0.062**	0.045	**0.075**	0.05
	Deltamethrin	0.077	0.06	0.1	0.5
YUVACIK [b] (2^{nd} sampling)	Azoxystrobin	1.1	0.829	1.371	15.0
GAZİVEREN[b]	Deltamethrin	0.02	0.015	0.025	0.5

[a]MRLs evaluated according to EU limits revised on 09/06/2005

[b]Villages where samples were taken in 2014 and 2015

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Molehiya is one of the traditional dishes in Northern Cyprus. The aim of this study was to determine pesticide residues in molehiya that is one of the most important minor crops of TRNC by using QuEChERS multi residue method.

In order to determine pesticide residues in molehiya, samples were collected from 22 regions in 2015 where 2014 sampling regions were also among these regions namely Bostancı (B), Ozanköy (O), Yuvacık (Y) and Gaziveren (G). Two molehiya samples in 2015, Bostancı and Ozanköy (O) out of 22 samples were containing pesticide residues.

As can be seen in the Figure 6, there was no any azoxystrobin residues in Yuvacık Molehiya samples any more. However, cypermethrin use in Bostancı region seems increasing and cypermethrin residues in Molehiya samples in 2015 were quite high; indoxacarb residues were also found in Bostancı molehiya samples and were again higher than MRL, although these molehiya samples were compliant after second presampling. There was no any problem related to indoxacarb use in Ozanköy region, whereas imidacloprid level was around MRL in the same region.

Although a MRL is the highest level of a pesticide residue that is legally tolerated in or on food or feed, it is not a level pointing out health risks, however it is an useful index to show whether good agricultural practices are applied or not in the field or in the orchard.

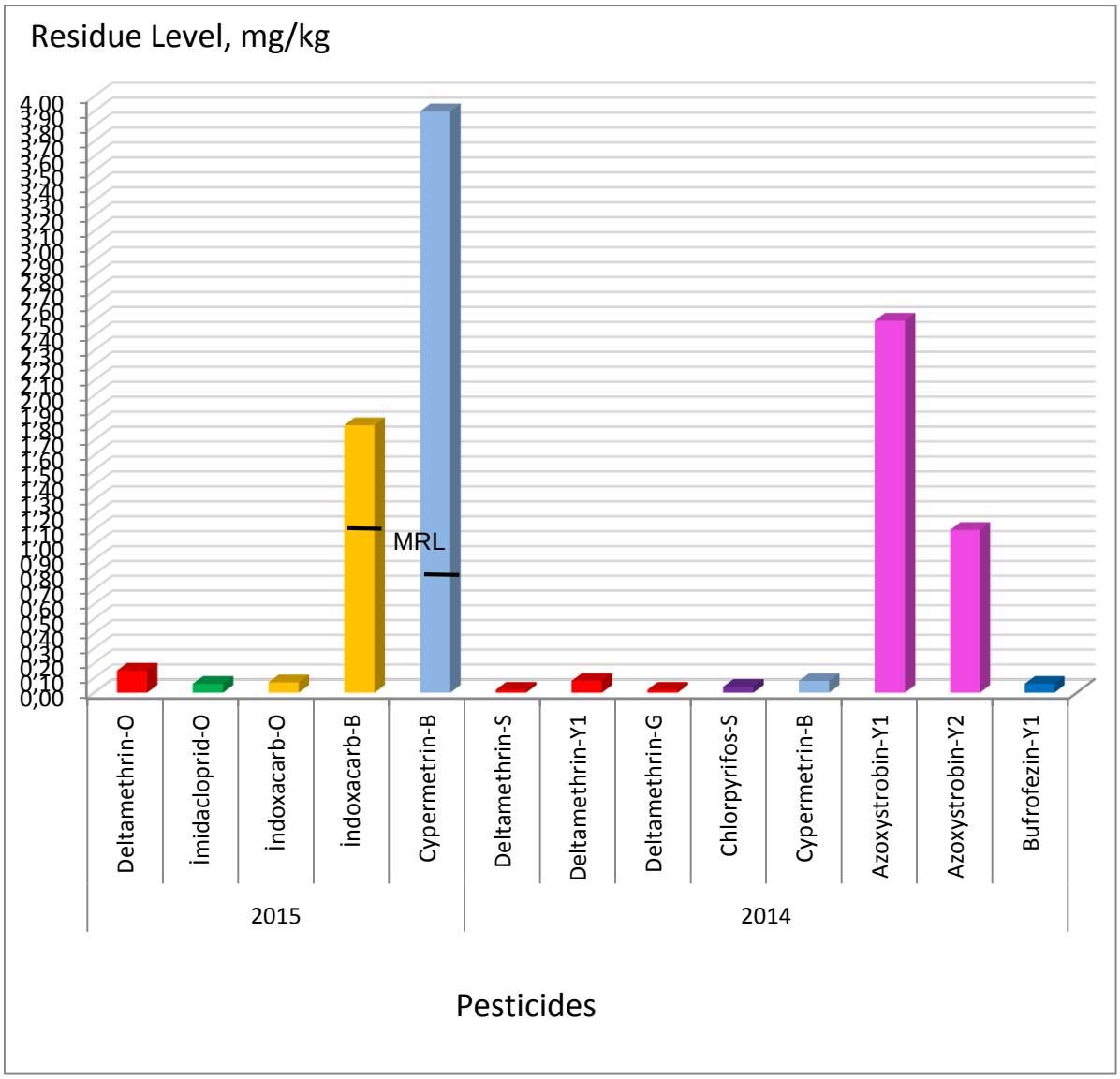

Figure 6: Pesticide residues detected in molehiya samples in 2014 and 2015

Therefore implementation of good agricultural practices in Bostancı and Ozanköy regions should be encouraged. Training of farmers and strenthening of monitoring and control programmes for safe use of pesticides are recommended for coming growing seasons.

REFERENCES

Ambrus, A. (n.d). Principles and practice of sampling. FAO/IAEA Training and Reference Centre for Food and Pesticide Control. Vieanna-Austria.

Anastassiades, M., & Scherbaum, E. (2004). Sample handling and clean-up methods II- new developments. In Fernandez-Alba (Ed.), *Comprehensive Analytical chemistry XLIII* (pp. 115-177). Elsevier B.V.

Bura, L. (n.d.). Testing the performance of the laboratories, training notes of FAO/IAEA training and reference center for pesticide and food control. Seibersdorf - Vienna, Austria.

Büyüksönmez, F., Rynk, R., Hess, T, F., & Bechinski, E. (1999). Occurrence, degradation and fate of pesticides during composting. *Compost Science & Utilization, 7*(4), 66-82.

Codex Alimentarius Commission. (1999). *Recommended methods of sampling for the determination of pesticide residues for compliance with mrls.* CAC/GL 33-1999. (pp. 1-18).

Codex Alimentarius Commission. (2003*). Guidelines on good laboratory practice in residue analysis.* CAC/GL, 40-1993, Rev.1-2003. (pp. 1-36).

Codex Alimentarius Commission. (2000). *Portion of commodities to which codex maximum residue limits apply and which is analyzed.* Codex Alimentarius, Volume 2A, Part 1-2000. (pp. 27-36).

Eşiyok, D., Kaygısız, T., & Bozokalfa, K. (2010). Sebze olarak değerlendirilen yeşillikler "Molehiya". Dünya Gıda Dergisi, Ağustos, 2010. Retrieved November 16, 2015 from http://www.dunyagida.com.tr/haber.php?nid=2275

European Commission. (2016). *Maximum residue levels.* Retrieved January 12, 2016, from http://ec.europa.eu/food/plant/pesticides/max_residue_levels/index_en.htm

European Food Safety Authority. (2015). *Pesticides.* Retrieved October 20, 2015, from http://www.efsa.euro-pa.eu/en/pesticides/mrls

European Standard. (2007). Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/ partitioning and cleanup by dispersive SPE - QuEChERS-method. prEN 15662:2007 (E).

FAO & WHO. (2008). *Principles and methods for the risk assessment of chemicals in food. Chapter 8: Maximum residue limits for pesticides and veterinary drugs.* Draft, May 2008. Retrieved August 10, 2015 from http://www.who.int/foodsafety/chem/residue_limits.pdf

García-García, C, R., Parrón, T., Requena, M., Alarcón, R., Tsatsakis, A, M., Hernández, A, F. (2015). Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. *Life Sciences,* from http://dx.doi.org/-10.1016/j.lfs.2015.10.013

Golge, O., Kabak, B. (2015). Evaluation of QuEChERS sample preparation and liquid chromatography–triple-quadrupole mass spectrometry method for the determination of 109 pesticide residues in tomatoes. *Food Chemistry, 176*, 319-332.

Hopper, M., & Boutrif. E. (2007). Strengthening national food control systems- A quick guide to assess capacity building needs. FAO of the United Nations. Nutrition Division. Rome: FAO/WHO.

İlhan, S., Savaroğlu, F., & Çolak, F. (2007). Antibacterial and antifungal activity of *Corchorus olitorius* L. (molokhia) extracts. *International Journal of Natural and Engineering Sciences 1(3),* 59-61.

İşeri, Ö.D., Yurtcu, E., Sahin, F.I., & Haberal, M. (2013). *Corchorus olitorius* (jute) extract induced cytotoxicity and genotoxicity on human multiple myeloma cells (ARH-77). *Pharmaceutical Biology, 51*(6), 766-770.

Jansson, C., Pihlström, T., Österdahl, B., & Markides, K.E. (2004). A new multi-residue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection. *Journal of Chromatography A, 1023*, 93-104.

Jicheng, Y., Qui, L., & Xiaohui, Q. (2011). Multiresidue method for the analysis of pesticide residues in leafy vegetables by supercritical fluid extraction and capillary gas chromatography. New Technology of Agricultural Engineering (ICAE), 2011 International Conference (pp. 1012-1017). Zibo: IEEE.

Joint FAO/ IAEA Programme. Nuclear techniques in food and agriculture: *Contaminants and residues*. Vienna: Austria. Retrieved December 05, 2015, from http://www-naweb.iaea.org/nafa/fep/topic-food-contaminants.html

Joint FAO/ IAEA Programme. Nuclear techniques in food and agriculture: *Food safety and traceability*. Food and Environmental Protection, FEP-NAFA. Vienna: Austria. Retrieved April 04, 2014, from http://www-naweb.iaea.org/nafa/fep/topic-food-safety.html

Kateman G. & Buydens L. (1993). Quality control in analytical chemistry, John Wiley & Sons Inc.

Kookanaa, R.S., Simpson, B.W. (2000). Pesticide fate in farming systems: Research and monitoring. *Communications in Soil Science and Plant Analysis, 31(11-14),* 1641-1659.

Lehotay, S.J., Kok, A.D., Hıemstra, M. & Bodegraven, P.V. (2004). Validation of a fast and easy method for the determination of 229 pesticide residues in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Dutch food and consumer products safety authority – Food inspection service, Region north west, R&D department, Pesticide analysis group; Hoogte Kadijk 401; 1081 BK. Amsterdam; The Netherland.

Li, J., Ying, Z., Rui, H., & Wei, P. (2011). Analysis of pesticide residues in vegetables from Shenyang, China. Intelligent Computation Technology and Automation (ICICTA), 2011 International Conference on, Volume: 2 (pp. 809-812). Shenzhen, Guangdong: IEEE.

Manual for IAEA by AGES. (n.d.). *Determination of pesticide residues by QuEChERS mini-multi method in samples of plant origin*. Document-No.: 1357-EN.

Marchis, D., Ferro, G.L., Brizio, P., Squadrone, S., & Abete, M.C. (2012). Detection of pesticides in crops: A modified QuEChERS approach. *Food Control, 25*, 210-273.

Mol, H.G., van Dam, R.C., Steijer, O.M. (2003). Determination of polar organophosphorus pesticides in vegetables and fruits using liquid chromatography with tandem mass spectrometry: selection of extraction solvent. *Journal of Chromatography A, 1015*, 119-127.

Oymen, B. (2014). Pesticide residues in ground water of Northern Cyprus. (Master dissertation), Near East University / The Graduate School of Applied Science, Nicosia.

SANCO. (2009). *Method validation and quality control procedures for pesticide residue analysis in food and feed.* Document no. SANCO/10684/2009, Implemented by 01/01/2010.

SANCO. (2013). *Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.* Document no. SANCO/12571/2013, Implemented by 01/01/2014. European Commission, health & consumer protection directorate-general. Retrieved September 16, 2015 from http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf

Sebze ve Meyve Sorunsuz. (2015, November 12). Kuzey Postası, Retrieved December 5, 2015 from http://www.kuzeypostasi.com/gundem/tarim-dairesi-muduru-emine-solyali-sebze-ve-meyve-sorunsuz/22396

Shujuan, J. (2011). Sample preparation methods for multiresidue analysis of pesticides by GC/MS in vegetables. *New Technology of Agricultural Engineering (ICAE), 2011 International Conference,* (pp. 810-813). IEEE.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods, 7th ed., The Iova State University Press.

Soboleva, E., Rathor, N., Mageto, A., and Ambrus, A. (2000). Estimation of significance of matrix-induced chromatographic effects. In A. Fajgelj, and A. Ambrus (Eds.), *Principles and practices of method validation* (pp.138-156). Bodmin, Cornwall, UK: MPG Books.

Sungur, Ş., & Tunur, Ç. (2012). Investigation of pesticide residues in vegetables and fruits grown in various regions of Hatay, Turkey. *Food Additives and Contaminants: Part B,* 5(4), 265-267.

Tarım Dairesi. Zirai Micadele ve Karantina Şubesi, (2014). *Yaprakları yenen yeşil sebzelerde ilaçlama programı – Yaprakları yenen yeşil sebzelerde hastalık ve zararlılarına karşı kullanılan bitki koruma ürünleri.* January, 2014. Lefkoşa: KKTC.

Tarladan Sofraya Yediklerimiz. (2014, February 28). Kıbrıs Gazetesi, Retrieved November 14, 2015 from: http://www.kibrisgazetesi.com/?p=169817

Tiryaki, O (2011). Pestisit kalıntı analizlerinde kalite kontrol (QC) ve kalite güvencesi (QA). Ankara: Nobel.

Tiryaki, O., & Temur, C. (2010). The fate of pesticide in the environment. *J. Biol. Environ. Sci.*, *4* (10), 29-38.

TRNC the Ministry of Agriculture and Natural Resources Statistic and Planning Division. (2010). *Agricultural structure and production 2009*. Lefkoşa.

Wilkowska, A., & Biziuk, M. (2011). Determination of pesticide residues in food matrices using QuEChERS methodology. *Food Chemistry, 125*, 803-812.

World Health Organization. (2015). *Pesticides residues in food.* Retrieved December 20, 2015, from http://www.who.int/features/qa/87/en

Yilmaz, N., Alas, T., & Abak, K. (2012). Collection of local vegetables genotypes along with their wild relatives in North Cyprus. *Vth Balkan symp. on vegetables and potatoes*. Acta hort. 960, ISHS 2012, (pp. 135-138).

Youden, W.J. J. (1967). Assoc. Off. Anal. Chem, 50. 1007-1013.

Yu, C., Fan, S., Li, Y., Zou, N., Li, X., & Pan, C. (2015). Representative commodity for five brassica vegetables based on the determination and dissipation of six pesticide residue**s**. *International Journal of Environmental Analytical Chemistry, 95*(5), 419-433.

Yücel. Ü. (1997). Klorpirifos'un topraktaki parçalanması. (Doctoral dissertation), Ankara Üniversitesi/Kimya Anabilim Dalı, Ankara.