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Determination of pesticide residues on tomatoes from greenhouses in Boudouaou and Douaouda, Algeria

I. Saidi[1*], F. Mouhouche[1] and H. Abri[2]

[1]Laboratory of Phytopharmacy, National Superior School of Agronomy, Hassan Badi Avenue, El Harrach, 16000 Algiers, Algeria; [2]Department of Chemistry, Saad Dahleb University, Road of Soumâa, BP270, Blida, Algeria; saidi-imene@live.fr

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RESEARCH ARTICLE

Abstract

The aim of this study was to assess the use of pesticides (insecticides and fungicides) on tomatoes grown in greenhouses in two important agricultural regions near Algiers, Algeria (Boudouaou and Douaouda). Twenty samples were taken (ten from each region) to measure the residual concentration of nine pesticides (chlorpyrifos-ethyl, fenitrothion, metalaxyl, procymidon, triadimenol, diazinon, lambda-cyhalothrin, cyfluthrin, and indoxacarb). Samples were analysed by the multi-residue method, with solid-phase-extraction purification followed by quantification using gas chromatography-mass spectrometry. Five pesticides were detected in tomato samples from the Douaouda area, including chlorpyrifos-ethyl (0.028±0.001 mg/kg), fenitrothion (0.68±0.004 mg/kg), metalaxyl (0.048±0.015 mg/kg), procymidon (0.046±0.02 mg/kg) and triadimenol (0.035±0.003 mg/kg). In the Boudouaou area, only three pesticides were detected: chlorpyrifos-ethyl (0.43±0.011 mg/kg), triadimenol (0.21±0.002 mg/kg) and procymidon (0.03±0.0055 mg/kg). Synthetic pyrethroids were not found in either region. These results provide important information on pesticide contamination of tomatoes in two key agricultural areas in Algeria, and highlight the need to monitor and control residual pesticide levels to protect consumer health.

Keywords: pesticide, GC-MS, Algeria, multi-residue analysis

1. Introduction

Over the last decade, agricultural practice in Algeria has undergone significant growth in terms of production, yield and the use of pesticides. These chemical compounds are regulated and approved by the Algerian Ministry of Agriculture; however, to maximise production, some farmers apply pesticides more frequently and heavily than is recommended. This is especially true for fruit and vegetable crops, because these are highly susceptible to diseases and attack by insects (Zawiyah *et al.*, 2007). As a result, residual pesticides appear in foods at levels that could affect consumers, particularly when the commodities are consumed raw. Residual pesticides are known carcinogens and toxins (Bempah *et al.*, 2011). Moreover, pesticides may have harmful effects on non-target organism. Major concerns include interference with reproductive systems and foetal development as well as the potential to cause cancer and asthma (Gilden *et al.*, 2010). In addition, the incorrect application of pesticides may induce resistance in the target organisms (Bakirci *et al.*, 2014).

The levels of pesticide residues on foods must fall below the maximum residue levels (MRLs) established by every country (Araoud *et al.*, 2007; Salghi *et al.*, 2012). In Europe, regulation (EC) no. 396/2005, modified by regulations (EC) no. 839/2008 and no. 459/2010 of the European Parliament and Council, established MRLs for pesticides in plant and animal products (EC, 2005, 2008, 2010). Monitoring pesticide residue levels is important to ensure that pesticides are not misused and that good agricultural practice are maintained (Keikotlhaile and Spanoghe, 2011). Of the many analytical methods developed to monitor residual pesticide levels in food, multi-residue methods are the most cost-effective (Yang *et al.*, 2011).

Tomato (*Solanum lycopersicul* L.) is one of the most consumed horticultural commodities in the world (Dorais *et* *al.*, 2008). In Algeria, it is considered an important vegetable because it is an essential component of many traditional meals. In 2008, in Algeria, attacks by the tomato pest, *Tuta absoluta*, caused enormous devastation to crops. Tomato production decreased significantly. To manage the pest, farmers used large amounts of different insecticides. Pesticide overuse almost certainly pushed residue levels above their MRLs. This is worrying because tomatoes can be eaten raw, which increases human exposure to pesticide residues. With these concerns in mind, the objectives of this study were:

1. To verify if pesticides targeted in this study are present on tomatoes collected from greenhouses in Douaouda and Boudouaou.
2. To quantify pesticide residue levels to compare these with established MRLs.

2. Materials and methods

Study site

This study focused on two important agricultural regions in the north of Douaouda and Boudouaou. Douaouda is 33 km to the southwest of Algiers, and Boudouaou is 35 km to the east of Algiers. These regions were chosen because of their high agricultural output; they provide produce to local markets throughout the year. In this part of Algeria, tomatoes are mostly cultivated in greenhouses, which may augment their susceptibility to pests and diseases. To mitigate this, many fungicides and insecticides (organophosphates and synthetic pyrethroids) are applied to the crops.

Selection of pesticides

To identify the most common pesticides used in our study regions, ten farms in each region were surveyed. Numerous criteria were evaluated by interviewing farmers. These included, general information about the owner of the farm (name, location, age) and the farm itself (nature of production and crops, area), as well as specific information about pesticide use. Survey data related to the chemical products were consolidated in a table, specifying the product name, its chemical class, the dose used, the application date, the number of treatments and the efficiency of the product.

Nine pesticides were determined to be the most commonly used compounds to treat tomato plants. These were found in both regions of study and included, fungicides and insecticides (organophosphates and synthetic pyrethroids). Table 1 lists the pesticides that were identified. All were approved by the Algerian authorities for use on tomatoes at the time of the study. All of these compounds were suitable for gas chromatography-mass spectrometry (GC-MS) analysis.

Sampling procedure

Once the study pesticides were defined, tomato sampling began. Twenty tomato samples were collected from the two areas (ten samples each). Samples were taken from different greenhouses, and blank samples were collected to calculate recovery. Sample sizes were limited to 1 kg, which is consistent with the sample sizes used in other work (Meredith and Jerry, 2002; Thier and Kirchhoff, 1992). The collected samples had to be homogeneous and representative of the plantation. Samples were placed in paper bags to protect them from light, which could otherwise have affected the products and target analytes. Samples were then transported to the laboratory and stored in a refrigerator at 5 °C until the time of analysis, the storage of samples at low temperature (5 °C) allowed to avoid any degradation that could affect target pesticides in tomatoes, in addition, the collected samples were analysed within 24 h of the time of their collection.

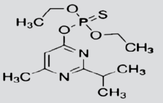
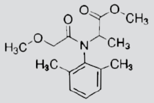
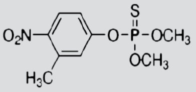
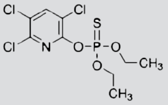
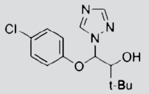
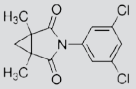
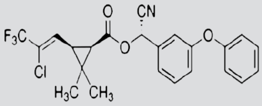
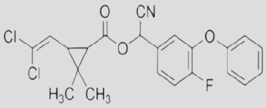
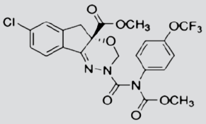
Sample processing

Multi-residue analysis is commonly carried out in a sequence of steps, including target extraction from the sample matrix, subsequent clean-up and pre-concentration, and, finally, chromatographic separation and molecular determination. Samples were blended using a food processor and mixed thoroughly. A homogenised subsample was weighed (to 15 g) and transferred into 250 ml centrifugation buckets before adding 30 ml of acetone. Samples were shaken well for 30 sec, and then 30 ml of dichloromethane and 30 ml of petroleum ether were added, followed by shaking for 30 sec. The buckets were then centrifuged for 10 min (4,000 t/min). After centrifugation, the supernatant (50 ml) was transferred to a flask and evaporated under vacuum using a rotator evaporator at a temperature below 50 °C. A 10 ml extract was subsequently obtained by mixing with solvents (90% isooctane and 10% toluene).

Clean-up is necessary to reduce the matrix effect, for this, solid-phase extraction (SPE) is a commonly employed technique (Stajnbaher and Zupancic-Kralj, 2003). Extraction was followed by a clean-up step using SPE employing a Florisil column (1 g; Resprep®; Restek Corporation, Bellefonte, PA, USA) activated with 5 ml of an acetone-hexane solvent mixture (1:1).

Pesticides in the sample extracts were then eluted with 5 ml of the same solvent mixture. The eluate was quantified by GC-MS. This method was chosen for its high sensitivity, selectivity and capacity to quantify a wide range of pesticides (Sharma *et al.*, 2010).

Table 1. The investigated pesticides and their chemical group/use, retention time (Rt), target ions and structure.

Investigated pesticides	Chemical group/use	Rt (min)	Target Ions (m/z)	Structure
Diazinon	organophosphate/insecticide	10.38	137, 179, 152	
Metalaxyl	phenylamide/fungicide	12.33	132, 160, 249	
Fenitrothion	organophosphate/insecticide	12.79	125, 109, 277	
Chlorpyrifos-ethyl	organophosphate/insecticide	13.54	97, 199, 197	
Triadimenol	triazole/fungicide	21.66	168, 128, 70	
Procymidon	dicarboximide/fungicide	21.96	283, 285, 67	
Lambda-cyhalothrin	synthetic pyrethroid/insecticide	27.13	181, 197, 208	
Cyfluthrin	synthetic pyrethroid/insecticide	32.17	206, 165, 227	
Indoxacarb	oxadiazine/insecticide	14.28	253, 203, 122	

Multi-residue analysis

A Perkin Elmer (Waltham, MA, USA) gas chromatograph coupled to a mass spectrometer was employed to conduct residue analyses. The apparatus was equipped with an auto-sampler and a capillary column with dimensions 30 m × 0.25 mm × 0.25 µm i.d. The column was an Elite-5 (5% diphenyl and 95% dimethylpolysiloxane; Perkin Elmer). Analyses were run with an injection temperature of 280 °C, and the following temperature programme: 2 min at the initial temperature of 70 °C, increase to 180 °C at 30 °C/min and hold 5 min, and finally increase to 310 °C at 25 °C/min and hold another 5 min. The total analysis time was 40 min.

Analyses performed in selected ion monitoring mode using one target and two or three qualifier ions. Target and qualifier abundances were determined by injecting pesticide standards under the same chromatographic conditions in full-scan mode with a mass/charge ratio (m/z) ranging from 50 to 500. This interval permitted scanning of a wide range of compounds. Pesticides were identified according to their retention time, the target and qualifier ions, and the qualifier to target abundance ratios. Quantification was performed using the internal software of the GC-MS.

To evaluate the efficiency of the analytical procedure, a recovery assay was employed. The method was validated by determining the limits of quantification (LOQ) and detection (LOD), the recovery percentages and the standard deviation.

The LOQ was defined as the lowest concentration of analyte that could be quantified with acceptable precision and accuracy, it corresponds to the analyte amounts for which the signal-to-noise ratio was 10, while the LOD was defined as the lowest concentration of analyte in a sample that could be detected but not necessarily quantified, it is determined as the analyte amounts for which the signal-to-noise ratio was 3 (Falqui-Cao *et al.*, 2001).

Reagents

Reagents (acetone and dichloromethane) were purchased from Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). All were HPLC grade. Hexane, toluene and petroleum ether were purchased from Biochem Chemopharma (Cosne Sur Loire, France). These reagents were also HPLC grade or specifically designated for the analysis of pesticide residues. Analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Riedel-de Haën (Seelze, Germany). Depending on the solubility of each standard, these were dissolved in 25 ml of either acetone or hexane to obtain a stock solution of 1000 mg/kg (1 g/l). Stocks were stored at -18 °C until used. Working solutions were obtained by diluting the stock solutions in hexane. These were prepared in a range of five concentrations ranging from 100 to 500 µg/kg (100 to 500 µg/l) and stored at 4 °C.

3. Results

In the present study, the LODs we obtained ranged from 0.0002 mg/kg to 0.005 mg/kg, and the LOQs ranged from 0.012 mg/kg to 0.025 mg/kg (Table 2). These are low and demonstrate the sensitivity of GC-MS for the analysis of pesticide residues in fresh vegetables.

At a fortification level of 500 µg/kg, average recoveries ranged between 90.61 and 103.36% (Table 2). These values all fall within the recommended intervals for the analysis of pesticide residues (70-120%; Fenoll *et al.*, 2007; Martinez Vidal *et al.*, 2002). Five pesticides were detected and quantified in tomato samples from Douaouda (Table 3). By contrast, only three were detected and quantified in samples from Boudouaou (Table 4).The detected pesticides were fungicides and organophosphates. No synthetic pyrethroids were detected in either region.

Table 2. Average recoveries (%), limits of detection (LOD), limits of quantification (LOQ), and R^2.

Pesticides	Average recoveries (%)	LOD (mg/kg)	LOQ (mg/kg)	R^2
Metalaxyl	99	0.005	0.025	0.995
Fenitrothion	90.61	0.005	0.025	0.95
Chlorpyrifos-ethyl	103.36	0.002	0.012	0.995
Triadimenol	118.61	0.03	0.05	0.98
Procymidon	94.96	0.0002	0.015	0.993

The region of Douaouda

Three fungicides were detected in all tomato samples from this region: procymidon, metalaxyl, and triadimenol. Five samples were contaminated with procymidon at residue levels that ranged from 0.046±0.015 mg/kg. Eight samples were contaminated with metalaxyl at residue levels that ranged from 0.048±0.015 mg/kg, and five samples were contaminated with triadimenol at levels that ranged from 0.035±0.003 mg/kg (Table 3), with one sample that exceeded the MRL (0.5 mg/kg). This contamination with fungicides may be a product of the sensitivity of tomatoes to fungal infection under greenhouses conditions, which promote the growth and development of fungi. Thus farmers used multiple fungicides to protect their crops. The sample with a pesticide level above the MRL is likely a result of non-professional use (Bai *et al.*, 2006). Either the farmers did not respect the recommended interval between treatments or they used excessive doses to protect the crop from diseases and insect attacks. This interpretation needs more investigation. Residues of the organophosphates fenitrothion and chlorpyrifos were also detected in tomato samples. Fenitrothion levels ranged between 0.68±0.004 mg/kg in three contaminated samples, whereas chlorpyrifos-ethyl residue levels ranged between 0.028±0.001 mg/kg in four contaminated samples (Table 3). All measured residue levels were below their recommended MRLs.

The region of Boudouaou

Fewer pesticides were detected in samples from this region than Douaouda (Table 3).Tomato samples collected in this area were characterised by the presence

Table 3. Detected pesticides, levels of contamination, and number of contaminated samples from Douaouda.

Detected pesticides	Average values (mg/kg) ± standard deviation	Number of contaminated samples	Number of samples exceeding MRL[1]	MRLs (mg/kg)[1]
Metalaxyl	0.048±0.015	8	1	0.5
Fenitrothion	0.68±0.004	3	–	1
Chlorpyrifos-ethyl	0.028±0.001	4	–	1
Triadimenol	0.035±0.003	5	–	1
Procymidon	0.046±0.002	5	–	NI

[1] MRL = maximum residue levels; NI = not indicated (Codex Alimentarius: http://tinyurl.com/h8r24wh).

Table 4. Detected pesticides, levels of contamination, and number of contaminated samples from Boudouaou.

Detected pesticides	Average values (mg/kg) ± standard deviation	Number of contaminated samples	MRLs (mg/kg)[1]
Chlorpyrifos-ethyl	0.43±0.011	6	1
Triadimenol	0.21±0.002	2	1
Procymidon	0.03±0.0055	4	NI

[1] MRL = maximum residue levels; NI = not indicated (Codex Alimentarius: http://tinyurl.com/h8r24wh).

of one organophosphate pesticide: chlorpyrifos-ethyl. This insecticide is widely used on tomatoes. Its residue levels ranged from 0.043±0.011 mg/kg, which are all under the MRL of 1 mg/kg as defined by the Codex Alimentarius. In addition to chlorpyrifos-ethyl, the fungicides triadimenol and procymidon were also detected and quantified (Table 4). Their residue levels ranged between 0.21±0.002 mg/kg and 0.03±0.0055 mg/kg, respectively. All values were under their respective MRLs.

The contaminated samples in both regions present potential health risks to consumers. Most of the samples contained more than one pesticide residue. This may be because the samples were cultivated in greenhouses, where they are very sensitive to pests and require consecutive pesticide treatments that leave considerable amounts of pesticide on plants. Moreover, under these conditions pesticides are protected from degradation by direct sunlight (Bempah *et al.*, 2011).

4. Discussion and conclusions

Sample preparation is often the most critical part of multi-residue analysis due to the diversity of substances extracted from vegetables (Chowdhury *et al.*, 2013).The extraction method used in this study was easily applicable, simple, fast, and robust. The method did not require large volumes of solvents. A mixture of solvents with different polarities was used to extract substances with different chemical characteristics, including polar and non-polar compounds. Dichloromethane, which we used here, is the most commonly used solvent for the extraction of polar to very polar pesticides (Ortelli *et al.*, 2004).

When analysing pesticides on fresh vegetables using GC, interference and contamination from plant pigments and the matrix are often encountered (Gang *et al.*, 2005); thus, a clean-up step is necessary because interfering substances in the matrix may co-elute with the target analytes (Martínez Vidal *et al.*, 2002). However, interference can usually be minimised using a column packed with Florisil (magnesium silicate, 60-100 mesh), as done in this study.

The optimised multi-residue method is suitable to analyse pesticides (fungicides and insecticides) approved for and commonly used on tomatoes in Algeria. Recoveries were good and the output showed good linearity, with R^2 values higher than 0.99 in most cases (procymidon, chlorpyrifos-ethyl and metalaxyl). The LODs and LOQs were low nd demonstrated the accuracy and the precision of GC-MS. Furthermore, the recommended clean-up step (SPE) compensated for any matrix effect that might have been associated with the samples. In our study, five pesticides were detected and quantified, indicating that the samples were contaminated. Residue levels were under their respective MRLs as set by the Codex Alimentarius, except for metalaxyl (in Douaouda). Nevertheless, the contamination levels provide important information on the current status of pesticide use in Algeria (Douaouda and Boudouaou) and highlight the need for monitoring and oversight to reduce the presence of these pollutants in foods and minimise health risks. Our work could be used by the regulatory authorities in Algeria to establish national MRLs and regulations to guide the use of pesticides on fruit and vegetable crops.

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