The persistence and dissipation of Chlorpyrifos in Lettuce in a greenhouse

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***Abstract*— This study describes two methods for determination of chlorpyrifos residues in lettuce by using gas chromatography / mass spectrometry and liquid chromatography / mass spectrometry. The experiment was conducted in a greenhouse near Durres. Chlorpyrifos emulsifiable was sprayed with dilute solution of *recommended* and double doses to the lettuce (*lactuca sativa*). Thesamples were collected every five days. Two different method for the same treated commodity shown that the concentration of the treatment with maximum recommended concentration were higher than European maximum residues levels. The methods gave satisfactory, fast and reliable results.**

Keywords—chlorpyrifos residues; dissipation; degradation curve; gas and liquid chromatography analysis

I. INTRODUCTION

The health benefits of eating fresh fruits and vegetables are well established, especially justified nowadays people have become aware about food safety. The use of toxic pesticides to manage pest problems has become a common practice around the world, but a primary concern in this process is the limited knowledge of farmers. The lack of proper information about pesticides is reflected in a survey conducted in 2015 in Albania, where 83.3% of farmers are interested in training courses on pesticide use, while 31.8% do not use Personal Protective Equipment (PPEs) [1].Applicators have three major responsibilities when applying pesticides in the field: protecting themselves, others, and the environment. Inappropriate application may result chlorpyrifos poisoning, which can affect the central nervous system, the cardiovascular system, and the respiratory system [2].

Pesticides should be used only if there is an economically important need and all pesticides must be used strictly in accordance with their label recommendation. Operators of spray equipment must receive appropriate training before handling and applying pesticides. The use of Personnel Protective Equipment (PPE) is essential for protecting operators' health. Directions and notices regarding how a given pesticide should be used can be found on the product label. Effective health monitoring records will be able to provide early warnings and identify changes in operator health, which may be attributed to working with pesticides [3]. Although the use of pesticides has many benefits in terms of crop and animal production, some of these pesticides and/or their metabolites or breakdown products are relatively toxic substances and could potentially have harmful effects on consumers of plant/animal products or the environment [4].

Most pesticide residues occur in food as a result of the direct application of a pesticide to a crop or farm animal or the post-harvest treatments of food commodities such as grains to prevent pest attack. Since the publication of Rachel Carson's book Silent Spring in the 1960s (Carson, 1965), there has been increased public concern about the impact of pesticides on the environment [5].

In this study, chlorpyrifos was used as foliar spray and according to directions of use on the product label. The degradation curve of chlorpyrifos residues was built from the day of treatment till the pre-harvesting interval (PHI). The evaluation of results were made using two different methods, one by using gas chromatography/mass spectrometry and another by using liquid chromatography/tandem mass spectrometry.

II. MATERIAL AND METHOD

A. *Experimental design*

The experiment was conducted in a greenhouse located in Hamallaj (Durres, Albania), with a surface area 5.000m^2 with respective coordinates N 41°28'26",E 19°32'57".The maximum and minimum recommended concentrations on the label of Chlorpyrifos (Terraguard Plus is the trade name) were applied during July 2015. The lettuce samples were collected regarding 2002/63/EC, approximately every 5 days after day of treatment.The dynamics of sampling was 1, 5, 10, 15, 17, 21 days after last application [6]. The 21th day corresponds to the pre-harvest interval, which is the period after the spray chlorpyrifos, residue remains on the lettuce.

B. *Chlorpyrifos residues by GC- MS/MS*

The sample extraction and clean-up were performed following the "SweET" method and analyzed by GC-MS/MS [7].

C. *Instrumentation, extraction and clean up*

Gas chromatograph: gas chromatography couple to tandem mass spectrometry (Agilent technologies 7890A) in multiple reaction monitoring (MRM) in EI MS/MS mode. The column used for pesticide residues separation with a column HP-5 MS (30 m x 250 μm x 0.2 μm). Mass spectrometer was operating in Electron impact Ionization (EI) mode. The electron energy was 70 eV and the temperature source at 290°C. The initial oven condition started at 50°C for 0,6 minute following by a temperature increase up to 180°C at a rate of 15°C/min held for one minute. Then the temperature was increased at 230°C by7°C/min and the last step was up to 280°C by 3°C/min. The injection volume was 1μL.

For extraction and clean up, from the homogenized lettuce, 10g was transferred to a 50mL falcon tube, and 20mL ethyl acetate was added, then the samples for 15 min were shaken on a mechanical shaker at 300 rpm/min. Subsequently, 10g sodium sulfate was added in each sample. The tubes were shaken again for 10 min and then centrifuged at 3200rpm for 3 min. An aliquot of extract was transferred into vials and then injected in the instrument [8].

D. *Gas chromatographic analysis*

The results of calibration curve and sample detection is shown below for the first method with GC-MS/MS.

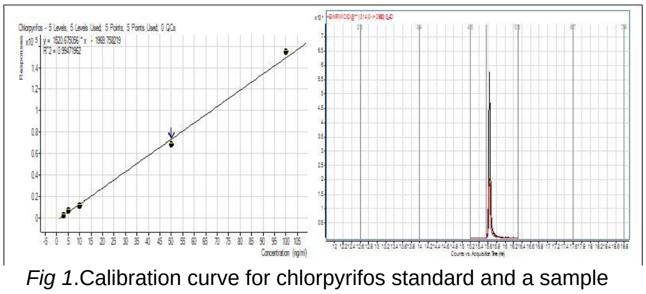

*Fig 1.*Calibration curve for chlorpyrifos standard and a sample chromatogram by GC MS/MS

E. *Chlorpyrifos residues by LC MS/MS*

The sample extraction and clean-up was performed following QuEChERS (EN 15662:2009) in laboratory of Food Safety and Veterinary Institute and the analysis by LC-MS/MS were performed at the Faculty of Agriculture, University of Novi Sad, Serbia.

F. *Instrumentation, extraction and clean up*

The LC-MS/MS analysis was performed on the Agilent 1200 HPLC system (Agilent Technologies, Waldronn, Germany) with an automatic degasser, a binary pump and an auto sampler connected to the Agilent 6410B Triple-Quad LC/MS system. The chromatographic separation was performed on the Zorbax XDB C18 analytical column of 50×4.6mm and 1.8 m particle size (Agilent Technologies, the USA), which was maintained at 30 °C.

The LC flow was maintained at 0.4 mL/min, the injection volume was 5 μL. The mobile phase gradient program started at 90% of B (water with 0.1% formic acid) and 10% of A (methanol with 0.1% formic acid) held for 2 min, then decreases to 10% at 15 and 5% at 17 min, held for 3 min. The mobile returned to the initial composition at 5.0 min and equilibrated for another 5 min before the next injection. Electro spray ionization was performed in the positive mode with the following parameters: resolution Q1 and Q3-wide (0.3 units) spray voltage-2000 V, gas temperature-325 °C, vaporizer-220 °C, gas flow (N2)-5 L/min; nebulizer gas (N2)-40 psi; the MassHunter software (version B.04. QQQ Agilent Technologies) controlled the LC-MS/MS system and processed the data.

For extraction and clean up, 10g of lettuce was homogenized and transferred in a 50mL PTFE centrifuge tube, 10mL acetonitrile was added and was shake for 1 minute, until uniform. Subsequently, QuEChERS citrate were added on each sample and shaken for 1min. Samples were centrifuged and the supernatant was taken for cleanup by adsorbent mixtures were added. Samples were shaken with the adsorbents of PSA then centrifuged to produce a clear supernatant for LC/MS-MS analysis [9].

E. *Liquid chromatographic analysis*

The results of calibration curve and sample detection is shown below for the second method with LC-MS/MS.

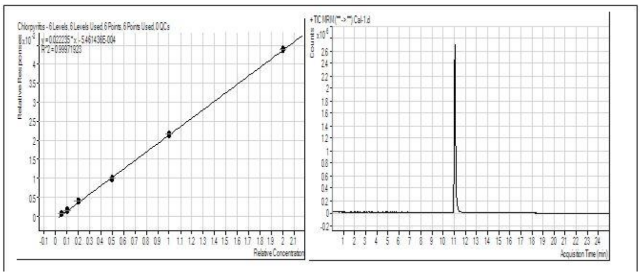

Fig 2. Calibration curve for chlorpyrifos standard and a sample chromatogram by LC MS/MS

III. RESULTS AND DISCUSION

A. The degradation curve of Chlorpyrifos was performed by two methods, by using gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. The rate of degradation of pesticide residue is affected by environmental conditions, nature of the pesticide, application rate, formulation, and plant species, etc. [10].

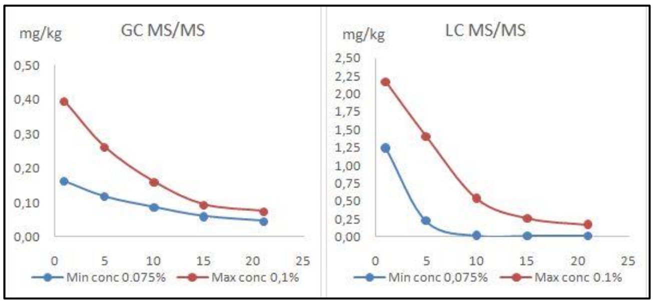

Fig 3. Dissipation dynamic of chlorpyrifos in lettuce

The first graphic describes the dissipation of chlorpyrifos with method SweET by GC-MS/MS and second graphic describes the dissipation of chlorpyrifos with Quechers method by LC-MS/MS. Both degradation curve show that for samples treated with the maximum recommended concentration need more days more for harvesting, because of the concentration values are above the maximum residues level MRL. While for the lower concentration of treatment chlorpyrifos is completely degraded before the day of PHI. The methods gave satisfactory results, fast and reliable in both cases.

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