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UHPLC-UHRMS and ^{109}AgNPs-assisted laser desorption/ionization mass spectrometry imaging of pesticide residues in *Solanum lycopersicum* L.

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ABSTRACT
Pesticide use in agriculture is a controversial topic due to concerns of health and environmental risks. Controlling plant diseases and crop quality with minimal negative impact on human and nature is of utmost importance. In this study ultra-high-performance liquid chromatography coupled with ultra-high-resolution mass spectrometry was employed for targeted pesticide detection of *Solanum lycopersicum L.* fruits from three different sources, revealing that the highest abundances of pesticides were present in a tomato from a local marketplace. Mass spectrometry imaging analysis of the most pesticide-affected sample was conducted, resulting in imaging four of the seventeen compounds detected by ultra-high-performance liquid chromatography-ultra-high-resolution mass spectrometry (UHPLC-UHRMS) analysis.

Keywords: pesticides, LC-MS, mass spectrometry imaging

1. Introduction

Plant protection products, commonly known as pesticides, are substances with a toxic effect to a certain group of organisms used in agriculture, water treatments and decontamination of buildings [1]. The main objective of pesticide use is providing produce of the highest quality and quantity crops, considering the ever-growing world population [2]. The main groups of plant protection products are insecticides, herbicides, fungicides and rodenticides. Organic insecticides are usually chloroorganic compounds, organophosphates, carbamates, pyrethroids, or neonicotinoids [2,3]. Compounds classified as herbicides include amides, phenoxy herbicides, bipyridine derivatives, dinitroanilines, pyrazoles, sulfonylurea derivatives, and triazines. In terms of fungicides usually used compounds are triazoles, benzenoids, morfolines, anilinepyrimidines, benzimidazoles, carboxamides, or ethylbenzamides [1]. Pesticides can also be classified by the effect on the targeted organism. The main categories are then systemic pesticides absorbed and distributed among the tissues of plants or targeted animals, such as 2,4,5-trichlorophenoxyacetic acid, pesticides active through contact which are not absorbed into the organism, for example paraquat. Another groups are substances active through digestion, such as malation, and fumigants which emit toxic gases, such as phosphine. A different class of substances are repellents, compounds that do not interact directly with the unwanted organisms. The example of such compound is diethyltoluamide, used to repel mosquitos [4].

The effect of pesticide use has its positive and negative impacts on human wellbeing and the environment. On the one hand, the use of pesticides allowed for decreasing the possibility of global world hunger with more crops of better quality by controlling many plant diseases. It can also positively influence the nutritional value and taste in certain cases [5]. On the other hand, pesticides can soak into soil and affect non-targeted plants or seep

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into groundwater and transfer to natural bodies of water, possibly poisoning and killing water animals [6]. Even if the active substance in the pesticide is seemingly not harmful to the environment, the degradation of the compound might lead to producing more toxic substances, such as the case of chlorpyrifos degrading to more toxic 3,5,6-trichloro-2-pirydynol [7]. In case of human health, plant protection products can interact with skin, breathed air, or consumption of food and water, and can cause both acute and chronic effects. Influence on the health of the affected person can range from light issues, such as skin irritation, dizziness, nausea, to coughs, chest and throat pain, sight impairment or fainting. The effects of long exposure to pesticide toxicity can cause memory loss, asthma and allergies, cancer, disruptions of the functionality of the reproductive system, Alzheimer's disease or epilepsy [8,9].

The objective of this study was to determine the use of plant protection products on common produce item, a tomato fruit. The comparison of the abundance of pesticides between samples obtained from different store types, including a local marketplace, a chain store, an a supermarket was conducted using the results of UHPLC-UHRMS. The sample with the most abundant amount of pesticides was then chosen for mass spectrometry imaging (MSI) to analyse the spatial distribution of detected pesticides in the epidermal or parenchyma part of the fruit. The importance of this topic is demonstrated by various studies concerning the effect of pesticides on human health and soil quality.

2. Experimental

2.1. Materials

Solanum lycopersicum L. or tomato fruits from a local marketplace (sample 1), a chain store Delikatesy Centrum (sample 2), and a supermarket Bi1 (sample 3) (all from Rzeszów, Poland) were used as samples for the analysis of pesticide residue occurrence. All used solvents were of LC/MS grade except for water (18 MΩ·cm water produced locally).

2.2. Methods

2.2.1. UHPLC-UHRMS of tomato fruit extracts

UHPLC-Q-ToF-UHRMS analysis was conducted with Bruker Elute UHPLC system, coupled with a Bruker Impact II mass spectrometer of ESI QToF-MS type. The UHPLC column used was the C18 Bruker Intensity Solo with silica functionalised with octadecyl groups, with 2 μm particles and dimensions of 100 × 2.1 mm (length × diameter). For mobile phases, water/methanol (99:1) with 5 mM ammonium formate and 0.01% HCOOH as phase A and methanol with 5 mM ammonium formate and 0.01% HCOOH as phase B were used. The injection volume was set at 5 μL, and the percentage of phase B were as follows: 4% (0–1 min), 99.9% (1.1-16 min), and 4% (16.1 - 20 min). From 0 to 2.5 min the solvent flow rate was 0.2 mL/min, from 2.6 to 19 it was 0.48 mL/min, and from 19.1 to 20 - 0.2 mL/min. To maintain consistent conditions, the UHPLC column was thermostated at 40°C during the analysis.

2.2.2. ^{109}AgNPs-LDI-MSI

^{109}Ag nanoparticles, as well as the target plate were prepared in accordance with our previously established procedure [10]. For the ^{109}AgNPs-LDI-MSI analysis, an imprint of a section of tomato including the pulp and epidermis was made on a stainless steel plate. Silver nanoparticles were sprayed onto the tomato imprint.

Measurements were performed using a Bruker Autoflex Speed time-of-flight mass spectrometer in reflectron mode. The apparatus was equipped with a Smart Beam II 1000 Hz 352 nm laser. Laser impulse energy was approximately 90–140 μJ, laser repetition rate was 1 kHz, and deflection was set on *m/z* lower than 95. Measured *m/z* range was 80–1500, experiments were made with 1000 laser shots per individual spot. All spectra were calibrated with the use of silver ions of $^{109}Ag^+$ to $^{109}Ag_{13}^+$ formula.

3. Results and discussion

3.1. UHPLC-UHRMS

The UHPLC-UHRMS analysis was performed using the TargetScreener option with build-in pesticide database. The goal of the analysis was to determine, which source of produce corresponded to the highest pesticide residue amount. The factors using for determining the most pesticide-contaminated tomato sample were the number of individual pesticides detected and the value assigned to the area under the curve. The results of the UHPLC-UHRMS were presented in Table 1.

Table 1. The results of the UHPLC-UHRMS analysis. The value of the area under curve was assigned for each pesticide found in three analysed tomato samples.

Pesticides		**Area**		
Name	**Type**	**Sample 1**	**Sample 2**	**Sample 3**
Azoxystrobin	fungicide	4176980	67209	416665
Boscalid	fungicide	53993	39431	-
Cyprodinil	fungicide	824787	-	-
Fenpyrazamine	fungicide	-	125820	-
Fluacrypyrim	acaricide	-	-	810743
Fludioxonil	fungicide	339920	20489	34810
Indoxacarb	insecticide	14970	-	-
Methoxyfenozide	insecticide	490887	-	-
Penthiopyrad	fungicide	316698	129666	-
Pyraclostrobin	fungicide	52171	20690	-
Spiromesifen	insecticide	-	-	31557
Spirotetramate-enol	insecticide	786948	55421	-
Spirotetramate-keto	insecticide	16931	-	-
Spirotetramate	insecticide	132065	-	-
Sulfoxaflor	insecticide	30523	-	-
Trifloxystrobin	fungicide	-	-	2180622
Zoxamide	fungicide	-	16824	227558

As evident by the results, the tomato fruit sample with the most individual pesticides, as well as the one found with the highest abundance of plant protection products was the sample obtained from the local marketplace. Although the sample bought at the supermarket also showed remarkable amount of few found compounds, the pesticides found in sample 1 were always of higher abundance where the comparison was possible. The results might feel counterintuitive at first glance, as locally bought produce has a reputation of the healthiest choice. The results however could present another perspective to the issue, where produce sold in stores have to pertain to rules and regulations of sustainable and health-driven agriculture laws more strictly.

3.2. ^{109}AgNPs-LDI-MSI

The previous UHPLC-UHRMS analysis also served as a preliminary choosing process for the MSI analysis. The sample with the most abundant occurrence of pesticides, sample 1, was chosen. Approx. 15 x 5 mm slice of tomato, including the pulp and the rind, was imprinted as stated previously. The outline of the imprint is visible in Fig. 1. Although pesticide detection was the main focus of this study, the identification of metabolites found in the sample was also possible with no additional steps. The imaging of compounds undeniably occurring in plant tissues, such as amino acids and other simple organic acids, provides additional confirmation, that the used imaging method could accurately present results in the imprinted area.

The ion images obtained for detected and identified metabolites show, that the method successfully differentiated between regions of compound occurrence. Compounds such as oxaloacetic acid (Fig. 1B) and geosmin (Fig. 1F) have been observed predominantly in the epidermal area of the fruit. Glutamic acid (Fig. 1C), galacturonic acid (Fig. 1H), pinocembrin (Fig. 1K), and gallocatechin (Fig. 1L) have been detected only in a small regions in the parenchyma, and the remaining 5 identified metabolites: adenine (Fig. 1D), histidine (Fig. 1E), lysine (Fig. 1G), pantothenic acid (Fig. 1I), and kinetin (Fig. 1J) were found in the whole parenchyma region in various abundances.

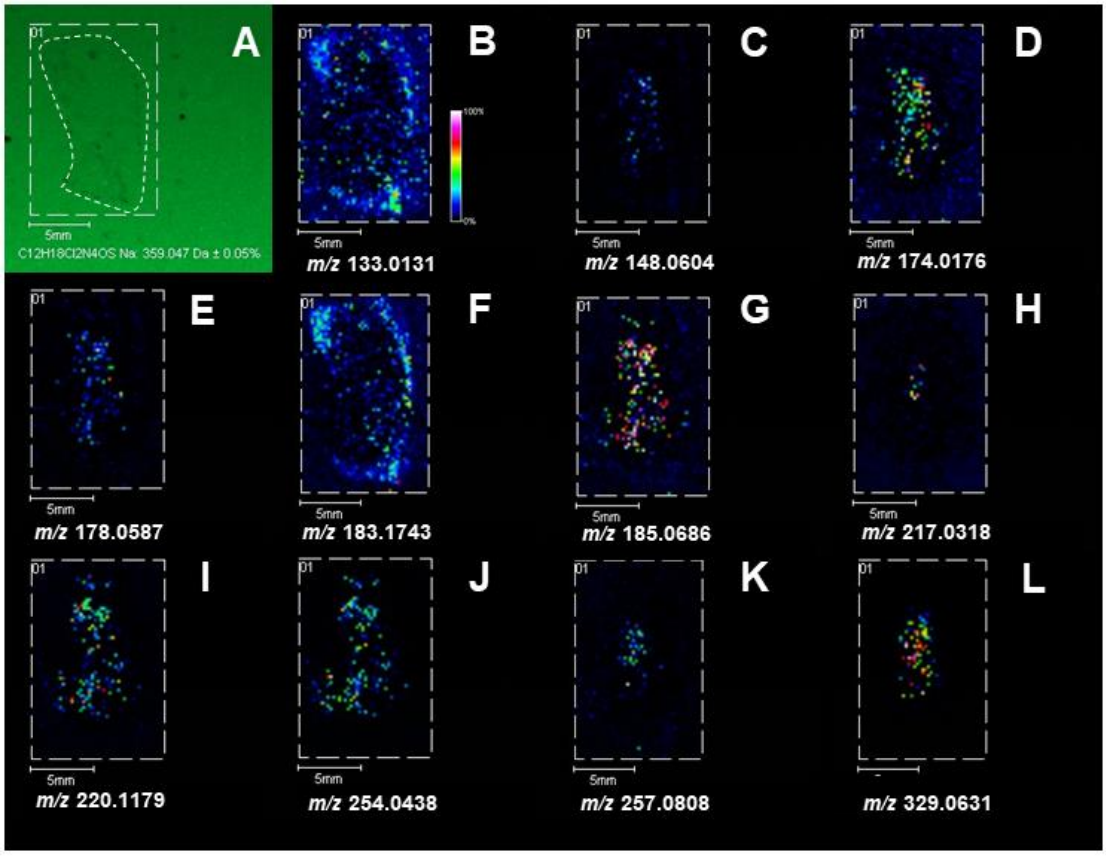

Fig. 1. Results of the detection of metabolites in the tomato section imprint. A – Outlines of the imprint on the target plate, B – [oxaloacetic acid + H]$^+$, C – [glutamic acid + H]$^+$, D – [adenine + K]$^+$, E – [histidine + Na]$^+$, F – [geosmin + H]$^+$, G – [lysine + K]$^+$, H – [galacturonic acid + Na]$^+$, I – [pantothenic acid + H]$^+$, J – [kinetin + K]$^+$, K – [pinocembrin + H]$^+$, L – [gallocatechin + Na]$^+$.

The MSI of the pivotal topic of the study, detection of pesticides in the tomato fruit, also provided results, confirming the occurrence of those compounds in the sample. Acquisition of ion images was possible for three of the plant protection products found in the marketplace sourced tomato by UHPLC-UHRMS analysis (Fig. 2). The first detected pesticide, fludioxonil was found in very low abundance. Nevertheless, slightly higher signals were observed in the imprint region, mostly in the parenchyma. Two different adducts provided ion images for boscalid distribution in the sample. Based on the abundance observed in the region of interest, relatively high levels of boscalid were detected in the parenchyma of the tomato fruit. A similar case was observed for penthiopyrad, based on ion image from one adduct. The last ion image obtained matched the m/z value of a pesticide not detected in sample 1 in the previous analysis. Trifloxystrobin occurred only in the supermarket sourced sample. The ion image also provides inconclusive result, as the potential pesticide's increased levels are indeed in the region of the imprint, however interpreting the image can be difficult when forming a definite conclusion. All of the detected plant protection products were fungicides, which generally both get absorbed into the plant tissues and protect them from the outside.

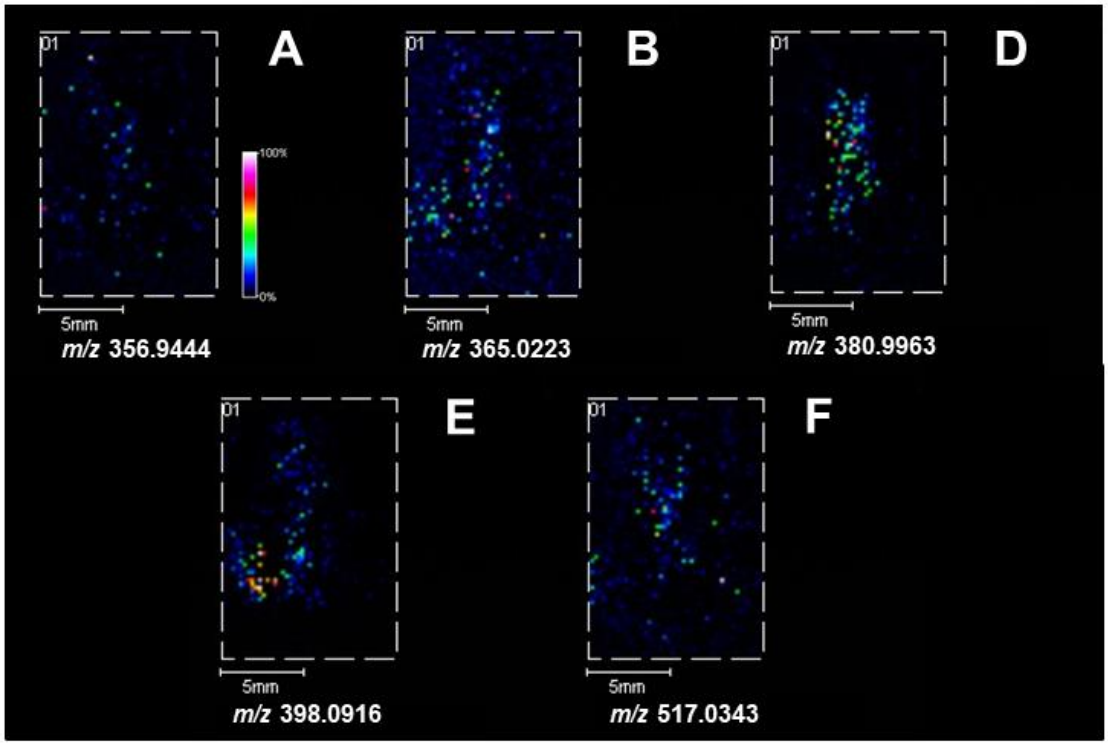

Fig. 2. The results of ^{109}AgNPs-LDI-MSI with the focus on detection of the previously identified pesticides. A – [fludioxonil + ^{109}Ag]$^+$, B – [boscalid + Na]$^+$, C – [boscalid + K]$^+$, D – [penthiopyrad + K]$^+$, E – [trifloxystrobin + ^{109}Ag]$^+$.

3.3. Conclusions

The occurrence of pesticides in tomato fruits obtained from different sources have been analysed. The results of UHPLC-UHRMS analysis allowed for detection of 17 individual pesticides across all samples, 12 in the tomato bought at a local marketplace, 8 in the sample from a chain store, and 6 pesticides in the tomato from a supermarket. 9 of the compounds were fungicides, 7 were insecticides, and one of them was an arachnicide. Undoubtedly the highest abundances of pesticides overall were detected in the plant sourced from the marketplace. Mass spectrometry imaging confirmed the existence of pesticides in the sample, also allowing for the analysis of spatial distribution of those compounds. Plant protection products were found solely in the parenchyma, and no ion image corresponding to the detected pesticides could be acquired of a pesticide occurring on the epidermal part of the fruit. Taking into consideration that fungicides indeed absorb through the tissue the results seem to allow for reaching reliable conclusions about the occurrence of plant protection products in the analysed sample. The abundance of pesticide residues in produce is often, and reasonably, associated with human health risks. However, other issues, such as soil contamination, sustainable agriculture, and food shortage problems should be taken into consideration when choosing the types and amounts of plant protection products used.

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