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Detection of Pesticides on Tomato Fruit Surface by Ultraviolet Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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Small amounts of pesticides and their transformation products may exist on edible parts before harvesting tomato (*Solanum lycopersicum* L.) fruits. For analyzing these compounds, special techniques with minimum preparations and high sensitivity are needed. The capability of a technique for in situ detection of target chemicals can be also a great advantage. Here we report the applicability of ultraviolet matrix-assisted laser desorption/ionization time of flight mass spectrometry (UV-MALDI TOF MS) for direct detection of pesticides and the residues on the tomato fruit surface. Fruits grown in the hydroponic system in a greenhouse were sprayed with a mixture of four pesticides including benomyl, triforine, milbemycin and malathion and collected one week later. The pericarp of sprayed and control fruits was peeled and located on a UV-MALDI plate, air-dried and covered with carbon nanotubes or 2,5-dihydroxybenzoic acid as matrixes. Signals of active and supplementary compounds which are normally present in commercial pesticides could be analyzed and directly detected on the surface of cuticle. A malathion degradation product was also detected on the sprayed fruit pericarp.

Keywords : *in situ* detection, malathion, pesticide residue, transformation product

INTRODUCTION

Pesticides are extensively used in agriculture throughout the world and have been remarkably contributing the crop yield and total food production. However, their residues on edible parts and the chemicals produced by the fragmentation/transformation of active ingredients are seriously taken into account in global trade, and many countries put strict limit of tolerance when importing or exporting food and food commodities. For example, in the case of tomato fruits in the United States, 5 and 8 ppm are the tolerance of benomyl and malathion, respectively (US Environmental Protection Agency, Office of Pesticide Programs, 2010). It can be expected that greenhouse-grown fruits may have greater amount of residues remained on the surface than field-grown fruits. The techniques able to analyze small amounts of residues directly on plant surface are of great importance in food chemistry and industry. Additionally, the fragmentation/transformation products of active ingredients and supplementary compounds found in commercial packages of pesticides may be hazardous to health. This complementary exploration contribute to find possible potential bio-

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hazards and can improve the quality of food and food commodities used by human and livestock. After spraying pesticides, residues and their fragmentation/transformation products on fruit may be in very low quantity. Therefore, high sensitive analytical techniques capable to detect pesticides are needed (Herrero et al., 2012). On the other hand, *in situ* detection techniques are of great importance since they provide the analysis in the original condition with a fast operation and minimum preparations. Hyphenated gas chromatography-mass spectrometry (GC-MS) methods have been long applied as the choice for the analysis of pesticide traces in food extracts (Alder et al., 2006; Botitsi et al., 2011). Recently direct detection of pesticides by mass spectrometry has been also introduced. By desorption electrospray ionization mass spectrometry (DESI MS), in which a charged (hot) solvent is sprayed on the surface with analyte, agrochemical traces have been directly analyzed (García-Reyes et al., 2009). For direct detection of agrochemicals, ambient pressure desorption ionization, in which a sample surface is exposed to a gas stream accompanied with a high-resolution mass spectrometer, has been also reported (Edison et al., 2011).

With its highly sensitive detector, ultraviolet matrix-assisted laser desorption/ionization time of flight mass spectrometry (UV-MALDI TOF MS) is a remarkable candidate for pesticide trace analyses, since it facilitates the detection of very low quantity of an analyte. Another interesting feature of UV-MALDI TOF MS is the possibility of the direct detection of chemicals on a surface without the need of specific preparations or extraction procedures. The only preparation in UV-MALDI MS of *in situ* biological tissues is the deposition of a suitable UV-MALDI matrix on the surface of the target tissue. UV-MALDI matrix is a compound capable to absorb UV laser photon energy and then to transfer this energy to analyte molecules which results in desorption and ionization of the analyte. However, availability of the suitable matrix is considered as one of the critical aspect of the technique as there are no clear criteria for the selection of a matrix for a specific analyte and the selection of matrix is empirical (de Hoffmann and Stroobant, 2007; Cole, 2010). The specificity and availability of matrix are more challenging when the analysis of chemicals on the surface of biological tissues is targeted. Some matrixes have shown to be able to desorb/ionize metabolites from the surface of plant tissue, including 2,5-dihydroxybenzoic acid (DHB) (Ng et al., 2007; Stahl et al., 1997), α-cyano-4-hydroxycinnamic acid (CHCA) (Ng et al., 2007; Robinson et al., 2007; Wu et al., 2007) and sinapinic acid (Ng et al., 2007). Nanoparticles including colloidal silver (Sluszny et al., 2005), and graphite (Zhang et al., 2007) have also shown to be useful for *in situ* plant tissue analyses. Carbon nanotubes (CNTs) and some metal nanoparticles (NPs) commercially available are capable to desorb/ionize chemicals from the surface of biological tissues (Gholipour et al., 2008; Gholipour et al., 2010). With CNTs, we were able to desorb/ionize metabolites from the surface of plant tissues (Gholipour et al., 2008). Application of CNTs and NPs has a great advantage; unlike organic matrixes, less chemical interaction between the matrix and the target tissue is expected (Gholipour et al., 2010).

Here, with DHB and CNTs as matrixes, we applied UV-MALDI TOF MS for *in situ* detection of pesticide traces and its transformation products on the surface of tomato fruit sprayed with a mixture of four commercial pesticides. The main goal of this work was to examine the applicability of UV-MALDI MS for detecting active ingredients, supplementary compounds and their fragmentation/transformation products on *in situ* fruit surface. The potential of the technique for relative quantitation will be also discussed.

MATERIALS AND METHODS

An aqueous solution of four pesticides (Table 1) including benomyl (Benelate, Sumitomo Co., Ltd., Japan), triforine (Sapurol, Sumitomo Co., Ltd., Japan), milbemycin (Chromite, Hokko Sangyo Co., Ltd., Japan) and malathion (Malathion, Sumitomo Co., Ltd., Japan) was prepared by mixing equal volumes of the commercial solutions of each, followed by diluting the mixture with water

(1:1,000 v/v). A diluted aqueous solution of each pesticide (1:1,000 v/v) was also separately prepared. Water of very low conductivity (MilliQ grade, 56–59 nS/cm with PURIC-S, Orugano Co., Ltd., Tokyo, Japan) was used for preparing the spray and matrix solutions. Tomato fruits (*Solanum lycopersicum* L. cv. Momotaro-8) were grown in a hydroponic system in a greenhouse with a day temperature of 25°C and night 20°C. A healthy plant was selected and was thoroughly sprayed with a garden sprayer with the mixture of four pesticides, while 3 fruits with a diameter of about 5 cm on the same plant were covered with nylon during spraying. Control and sprayed fruits (3 fruits per treatment with similar diameter) were collected one week later. On intact outer pericarp tissue of each fruit, 2 slices (5 mm by 5 mm) were made with a razor blade. No further treatment was made on slices. The tissue samples were then located directly on a gold-covered, 100-well MALDI plate (part number V700401, Applied Biosystems, USA). Matrixes including carbon nanotubes (CNTs, suspended in an aqueous 50% ethanol solution), 2-(4-hydroxyphenylazo)-benzoic acid (HABA) and 2,5-dihydroxybenzoic acid (DHB) dissolved (4 mg) in methanol and water (0.5 mL and 0.5 mL) were deposited (2 μL) on fruit peels and air-dried with an air blower at room temperature for about 1 hour. To avoid the effect of fruit to fruit variation in comparing the efficiency of matrixes, slices from the same fruit covered with two matrixes were analyzed. Individual pesticide solutions were pipetted (1 μL) on layers of matrixes previously deposited (1 μL) on the plate and air-dried at room temperature for few minutes.

Analysis was carried out on a Voyager-DE STR time-of-flight mass spectrometer utilizing pulse delayed extraction (PDE, 125 nsec) in linear positive and negative ion modes. Desorption/ionization was obtained by using a 337 nm nitrogen laser with the 3 ns pulse width, an accelerating potential of 20 kV and a 95% grid voltage. On average, 100 shots were recorded per sample. The lowest laser power that yielded a high signal-to-noise (S/N) ratio with a suitable resolution of peaks of the analyte was utilized. External calibration was carried out by using standard mannitol and β-cyclodextrin (Wako Chemicals, Japan) deposited on CNTs and DHB. For the verification of signals directly detected on the cuticle surface, *in situ* tissue mass spectra were compared with the mass spectra of individual pesticides deposited on the same matrix. Mass spectra were analyzed with Data Explorer® software ver. 4.1 (Applied Biosystems, USA). For further verification, chemical formula of favorite signals was simulated by using Elemental Composition applet in Data Explorer® software.

RESULTS AND DISCUSSION

Analyses of pesticide solutions

When analyzing pesticide solutions deposited on air-dried matrix, among examined matrixes, pesticide signals were detected only with CNTs and DHB. In addition, the analysis was successful only in the positive ion mode as no pesticide signal was detected in negative mode (results not shown). Figure 1 shows positive ion mode mass spectra of individual pesticide solutions with CNTs as the matrix. Benomyl was detected as a sodiated signal with m/z 313.13 (Fig. 1B). Furthermore, several unidentified peaks arising from compounds in Benelate commercial solution were also detected. However, no significant surfactant signals were appeared. Triforine with m/z 434.93 (protonated ion) was detected in Sapurol mixture (Fig. 1C). From Sapurol solution, a series of polymeric peaks with 14 m/z intervals (e.g. m/z 460.9, 475.9, 489.9, 502.9 etc.) were obtained in the range of m/z 300–800. The 14 Da repeating unit, i.e. —[CH_2]—, can be attributed to alkane chains of waxes or fatty acids (Tong et al., 1999). Since these peaks originated from compounds in a pesticide solution, it could be assumed that those signals were from surfactant(s) which is commonly added to commercial solutions of pesticides. With CNTs, we could not detect any signals related to milbemycin in Chromite solution (Fig. 1D). However, in the m/z range of 300–1200, two series of polymeric signals with a relative distance among their compounds of 16 m/z units (e.g.

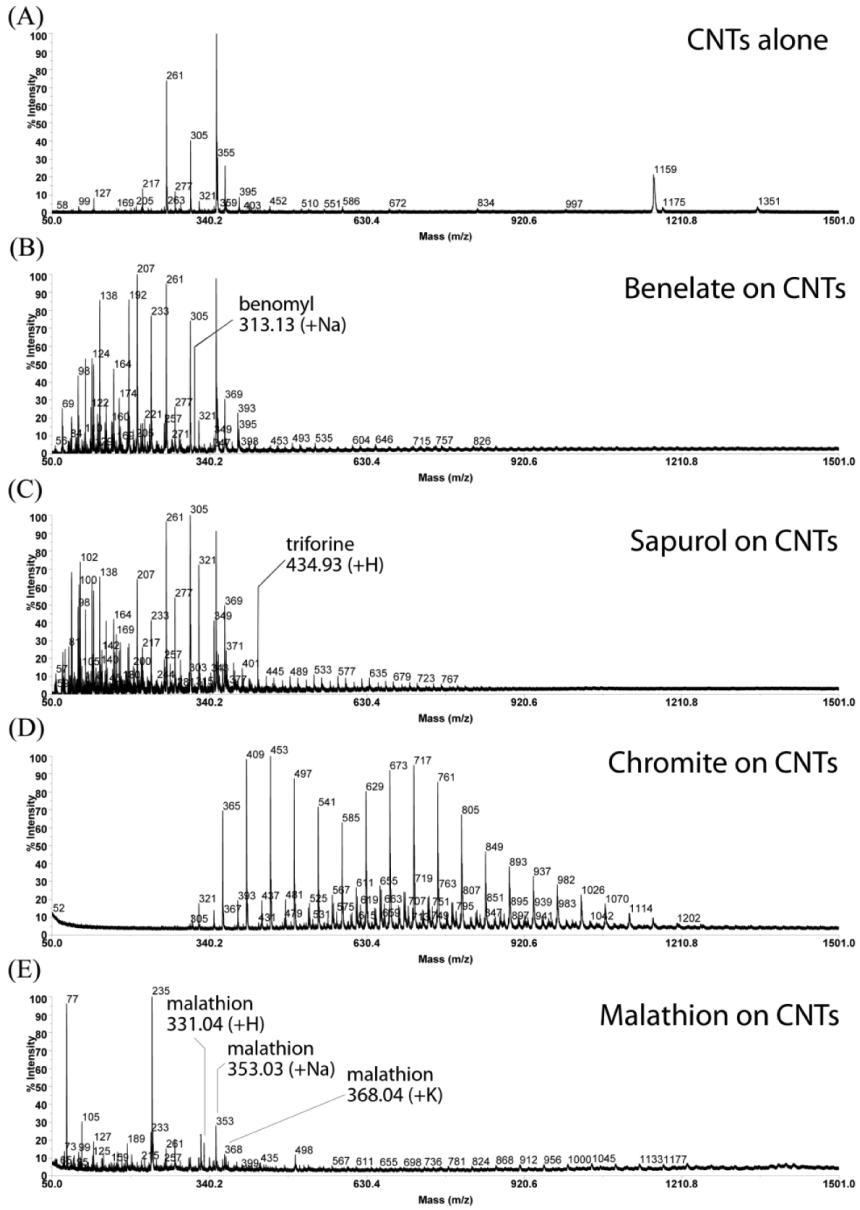


Fig. 1 Positive ion mode UV-MALDI mass spectra acquired after depositing commercial solutions of the pesticides on an air-dried layer of CNTs on a gold MALDI plate. A: CNTs alone, B: Benelate on CNTs as matrix, C: Sapurol on CNTs as matrix, D: Chromite on CNTs as matrix, E: Malathion on CNTs as matrix.

m/z 364.7, 408.7, 452.7, 496.7 and m/z 348.7, 392.7, 436.7, 480.7 etc.) were detected. The relative distance among the peaks in each series was 74 m/z units. In the positive ion mode, signals with 44 Da intervals have been attributed to polyethylene glycol or related detergent compounds such as triton (Tong et al., 1999). The signals in the second series may be the oxidized form of the first series molecules. In the case of the malathion solution deposited on CNTs, malathion signals were detected as $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ with the m/z values of 331.04, 353.03, and 368.04,

respectively (Fig. 1E). Here, a series of small peaks with 44 m/z units intervals also appeared.

Unlike CNTs, DHB matrix was able to yield the signals of active ingredients of all examined pesticides (Fig. 2). Benomyl with protonated signal of m/z 291.15 and sodiated signal of m/z 313.13 were detected (Fig. 2B). Polymeric signals with repeating m/z units of 44 appeared in the mass spectrum of Benelate deposited on DHB. Triforine, the active ingredient of Sapurol pesticide,

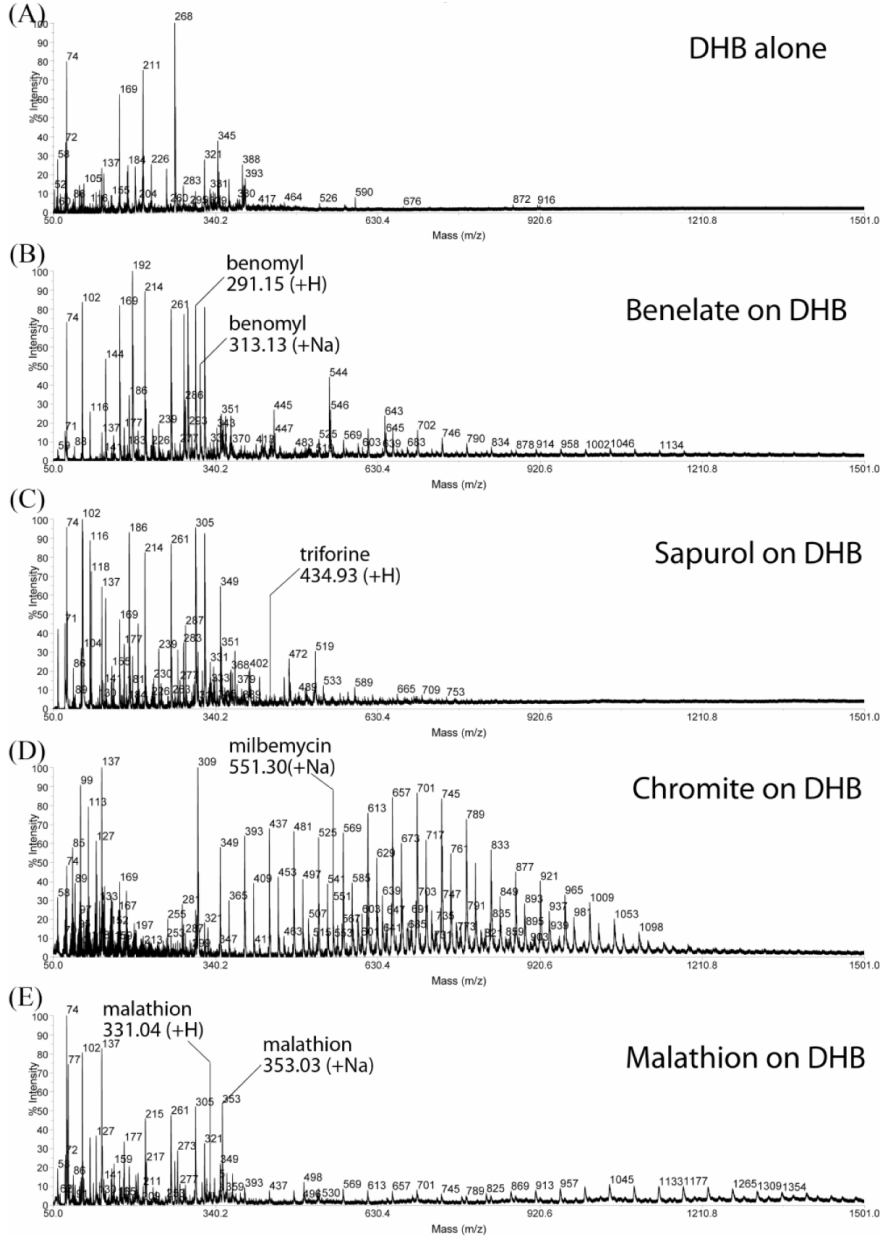


Fig. 2 Positive ion mode UV-MALDI mass spectra acquired after depositing commercial solutions of the pesticides on an air-dried layer of DHB on a gold MALDI plate. A: DHB alone, B: Benelate on DHB as matrix, C: Sapurol on DHB as matrix, D: Chromite on DHB as matrix, E: Malathion on DHB as matrix.

was detected as a protonated (m/z 434.93) peak (Fig. 2C). Unlike CNTs, no remarkable polymeric signals were appeared in the Sapurol solution deposited on DHB. In case of Chromite, milbemycin yielded a signal with m/z 551.30, $[M+Na]^+$ (Fig. 2D). Also, polymeric peaks with 44 m/z units intervals appeared. The protonated and sodiated ions of malathion with m/z of 331.04 and 353.03 were detected (Fig. 2E). In mass spectra of the malathion solution deposited on DHB, polymeric peaks with an interval of 44 m/z units appeared.

Sub-femtomoles of active ingredients have been deposited on the MALDI plate (Table 1). Therefore, the technique showed capability for detecting and analyzing very small amount of pesticides.

UV-Laser desorption/ ionization MS experiments

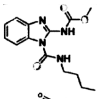
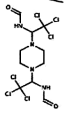
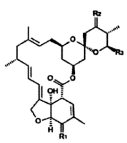
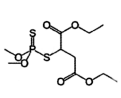
Provided analyte molecules interact with UV laser desorption/ ionization (UV-LDI) MS can be a great analytical technique since matrix molecules are not needed any longer. Consequently, preparations and interfering signals from matrix and solvents are minimized. We irradiated 337 nm UV laser directly on the control and sprayed cuticles. Some peaks appeared in the sprayed cuticle (Fig. 3) but they could not be detected in MALDI mass spectra of pesticides deposited on CNTs and DHB.

A reliable signal assignment could not be performed for LDI MS results since their mass and elemental composition did not match to any compounds in the NIST 11 mass spectral library. The lack of results can be explained because the studied compounds, whose structures are shown in Table 1, do not absorb at 337 nm.

UV-MALDI MS analyses of pesticide residues

In all samples taken from control and sprayed fruits, signals of pesticide ingredients were ob-

Table 1 List of examined pesticides with their active ingredients.

Active ingredient	M Formula (molecular weight, Da)	M Structure	M+X (Detected ion m/z)	Commercial package	Amount of the active ingredient (fmol)	
					in 1 μL of diluted pesticide solution deposited on each matrix spot on the MALDI plate	in 1 μL of diluted mixture solution sprayed on tomato plants in a greenhouse
benomyl	$C_{14}H_{18}N_4O_3$ (290.14)		M+H (291.15) +Na (313.13)	(50%), Benelate, Sumitomo Co., Ltd., Japan	1700	431
triforine	$C_{10}H_{14}C_{16}N_4O_2$ (433.92)		M+H (434.93) M+Na (456.91)	(15%), Sapurol, Sumitomo Co., Ltd., Japan	346	86
milbemycin	$C_{31}H_{44}O_7$ (528.31)		M+H (529.32) M+Na (551.30)	(1%), Chromite, Hokko Sangyo Co., Ltd., Japan	19	5
malathion	$C_{10}H_{19}O_6PS_2$ (330.04)		M+H (331.04) M+Na (353.04) M+K (369.03)	(5.5%), Malaton, Sumitomo Co., Ltd., Japan	167	42

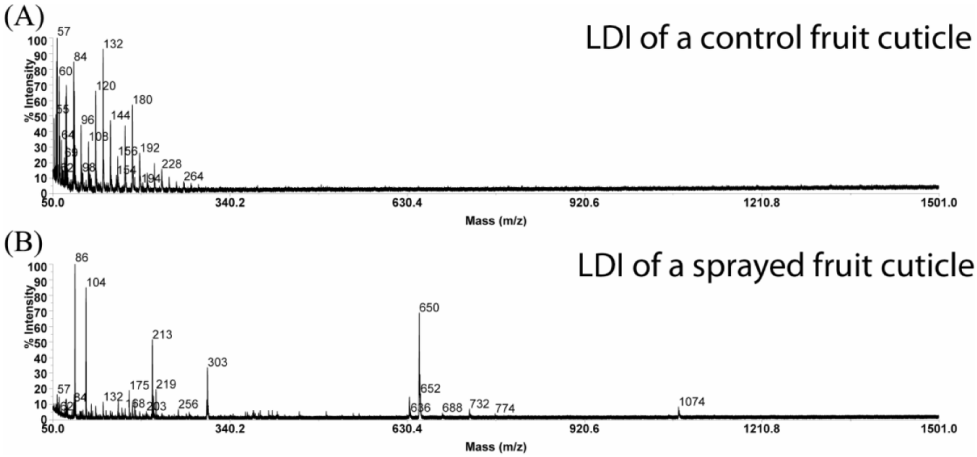


Fig. 3 Positive ion mode UV-LDI mass spectra acquired by irradiating UV laser directly on the tomato fruit pericarp slices without using any matrix. A: Control without matrix, B: Pesticides sprayed without matrix.

served. However, the number of peaks and their signal intensity were not necessarily same for all samples. Here, mass spectra containing more information and higher signal intensity peaks are shown. With CNTs deposited on the tomato fruit pericarp, signals originated from pesticide solutions appeared (Fig. 4B). Among active ingredients, malathion (as the sodiated peak with m/z of 353.04 and potassiated of m/z 369.03) and triforine (sodiated peak of m/z 456.91) were detected. By shooting the UV laser onto the pericarp surface covered with CNTs, several peaks previously detected in the pesticide solutions were identified, as well.

With DHB deposited on a sprayed fruit pericarp, malathion peak (m/z 331.04, $[M+H]^+$) with several other peaks originated from the pesticide solutions was detected (Fig. 5B). Unlike CNTs with which no polymeric peaks could be yielded, with DHB as the matrix two series of polymeric signals with a relative distance among their peaks of 16 m/z units and of 70 m/z units intervals between peaks in each series were detected.

Abundant triforine and malathion peaks showed that these active ingredients were persistent on the tomato cuticle even one week after spraying. Importantly, we detected their traces on an edible part. It has been shown that after entering into the human body, malathion translocates to the liver and kidneys and finally affects the nervous system (National Pesticide Information Center, 2011).

The number of molecules of active ingredients in the spray mixture solution varied between 5 to 500 fmol/μL (Table 1). It can be assumed that each slice area had been covered with about 2 μL of pesticide solution during spraying fruits in the greenhouse. Since analysis was performed several days after spraying, it can be concluded that with our technique atto- to femtomoles of pesticide residues could be detected on plant and fruit surface with UV-MALDI MS analyses.

Pesticides transformation products

In mass spectra acquired by depositing CNTs and DHB on a sprayed cuticle, several new signals were observed (Figs. 4 and 5). One of strong possibilities on the origin of such signals is that they would be some transformation and fragmentation products of the original ingredients (Botitsi et al., 2011). These products can be more toxic than the parent pesticide (García-Reyes et al., 2009; Richardson, 2008). A few reports on the transformation and fragmentation products of pesticides are available and therefore, in this work the verification of those signals was carried out only for malathion.

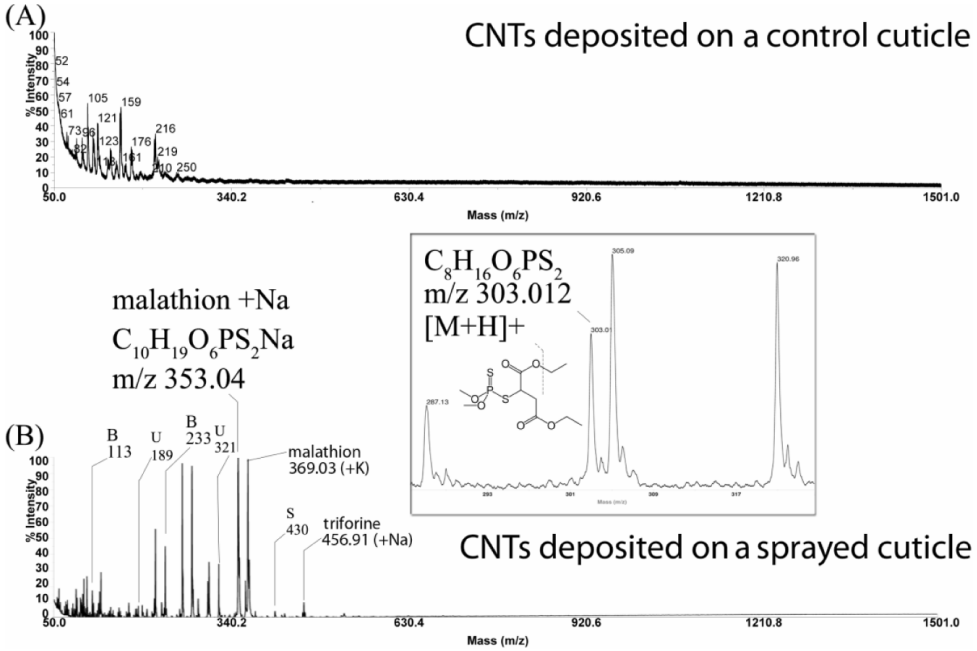


Fig. 4 Positive ion mode UV-MALDI mass spectra acquired after depositing CNTs on the pericarp of the control (A) and sprayed tomato fruits (B). In (B), S, C, M and U represent signals originated from Benelate, Sapurol, Chromite and Malathion solutions, and unidentified compound, respectively. Inset in (B) shows an identified malathion degradation product.

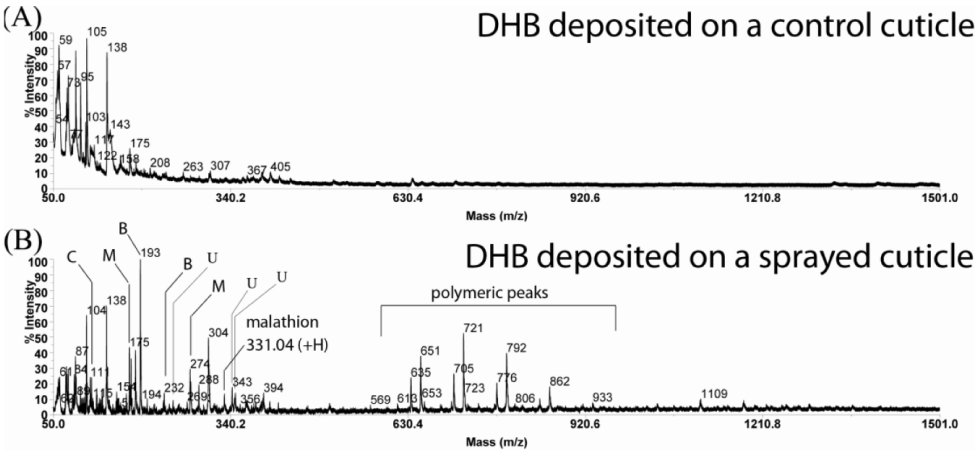


Fig. 5 Positive ion mode UV-MALDI mass spectra acquired after depositing DHB on the pericarp of the control (A) and sprayed tomato fruits (B). In (B), S, C, M and U represent signals originated from Benelate, Sapurol, Chromite, Malathion solutions, and unidentified compound, respectively.

We detected a malathion transformation product (Fig. 4B inset). This compound with m/z of 303.01 has been proposed to be produced by an ester hydrolysis of an ethyl group as shown in Fig. 4B inset (García-Reyes et al., 2009). Additionally a series of polymeric peaks of unknown structure were found (Fig. 5B) which we assumed that they were also transformed molecules of original detergents or surfactants present in commercial products. It has been elucidated that malathion can

produce more toxic products if persists for a longer time, especially in warm environments such as greenhouses (National Pesticide Information Center, 2011). Since tomatoes were grown at the room temperature and it persisted on the plant surface, our conclusion on the existence of its transformation products is consistent with the literature (García-Reyes et al., 2009; National Pesticide Information Center, 2011).

The advantages of the *in situ* UV MALDI MS technique are the least preparation and the easy-to-do and fast analysis capability. UV-MALDI MS has become a crucial technique in contemporary biological analyses. In this work we showed that this technique is applicable to the direct detection of agrochemical residues on the surface of fruits. Therefore, it is worthy to conduct more experiments in the future to achieve lower limit of detection and the analysis of larger number of pesticides and their transformation and fragmentation products. There is the possibility of quantitative analysis of pesticide residues with UV-MALDI mass spectrometry. First, signal abundance of residues and their fragments can be used for comparing relative difference in the amount of residues on the surface of fruits. Secondly, known amounts of pesticides can be deposited on control fruits and their signal abundance can be compared with those of residues. For comparative purpose pesticides residues can be collected from the surface by a suitable solvent using well established protocols and then, they can be quantified by using liquid or gas chromatography-mass spectrometry (LC-MS or GC-MS) techniques (Alder et al., 2006; Botitsi et al., 2011). Afterwards information obtained by LC- or GC-MS can be used for better interpretation of results obtained by direct UV-MALDI MS analysis of tissues.

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