Rapid and Nondestructive Detection of Pesticide Residues by Depth-Profiling Fourier Transform Infrared Photoacoustic Spectroscopy

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ABSTRACT: Detection of pesticide residues is important for ensuring food safety, and it has assumed increased significance. Traditional analytical methods are known for being destructive and cost- and time-intensive. In this study, depth-profiling Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) was successfully used as an in situ, nondestructive, and rapid method for detecting tricyclazole residues on three metal surfaces (copper, aluminum, and iron) and subsequently, on the surfaces of fresh rice leaves and ripe husks. Four moving mirror velocities, that is, 0.32, 0.63, 0.95, and 1.90 cm s⁻¹ were used for recording the spectra. The results indicated that the moving mirror velocity of 0.95 cm s⁻¹ was optimal for depth profiling, and the obtained spectra showed a strong absorption band at around 1200 cm⁻¹, corresponding to the C–N bond in tricyclazole. This band could be used for monitoring tricyclazole residues on plant surfaces. Principal component analysis confirmed the detection of tricyclazole on the basis of its spectral information. Considering the scanning depth and the thickness of the plant cuticle, FTIR-PAS can be an effective means for detecting and monitoring similar organonitrogen pesticide residues.

INTRODUCTION

Pesticides, which are agrochemicals, designed to prevent, destroy, repel, or mitigate pests,¹ have played a significant role in facilitating the development of agriculture.² However, their inappropriate and irrational use has resulted in an increase in the amount of pesticide residues on plants, vegetables, and fruits, which adversely impact the ecosystem and human health.³,⁴ Consequently, many countries began focusing on pesticide control and detection of pesticide residues,⁵ using a range of analytical techniques such as gas chromatography-mass spectrometry,⁶ high-performance liquid chromatography,⁷ enzyme inhibition method,⁸ enzyme-linked immunosorbent assay,⁹ and electrochemistry.¹⁰ Despite their wide usage, these traditional methods are known to be time- and cost-intensive and require complicated pretreatment processes to facilitate detection. Therefore, a rapid, inexpensive detection technique is needed.

Infrared spectroscopy is an effective tool for qualitative and quantitative analysis¹¹ and has already been deployed for pesticide determination in agrochemical formulations.¹² Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS), consisting of a movable mirror whose velocity can be changed, has been used for depth profiling in a wide range of fields.¹³–¹⁵ The analyte molecules absorb electromagnetic radiation, leading to local warming, and the thermal expansion generates pressure fluctuations, which are detected by a sensitive microphone and finally transformed into spectral data.¹⁶–¹⁸

The obtained spectrum contains abundant information about the sample, and the detected signal is proportional to the sample concentration.¹⁹–²¹ Armenta et al.²² managed to apply FTIR-PAS for the routine determination of mancozeb in commercially formulated fungicides, and Liu et al.²³ coupled PAS with an artificial neural network method to non-destructively quantify dichlorvos residues on the apple cuticle in the near-ultraviolet region. Although there have been few reports about its application in detecting pesticide residues, FTIR-PAS in the middle wavelength range shows stronger potential for the quantification of pesticide residues on the surface of plant cuticles.

The plant cuticle is the outer protective layer on the surfaces of aerial plant organs,²⁴ and it is a layered structure composed of epicuticular wax, intracuticular wax, and cuticle matrix.²⁵ The main components of cutin and cuticular waxes are fatty acids, alcohols, aldehydes, alkanes, esters, and some other lipophilic polymer matrices and soluble cuticular lipids.²⁶ As a thin, hydrophobic, and flexible membrane (measuring 0.1–10 μm in thickness), the cuticle is an efficient barrier against unregulated water loss and pathogen infection.²⁷ Organonitrogen pesticides, comprising several nitrogenous compounds, are some of the most universally administered pesticides because of their low...
cost, ready availability, and wide applicability. Tricyclazole is one such fungicide effective against the fungus *Magnaporthe oryzae*, known to cause the blast disease. It consists of a five-membered heterocyclic ring with three nitrogen atoms (Figure 1), which forms the basis for determining organonitrogen pesticides by FTIR-PAS.23

This study aimed to apply depth-profiling FTIR-PAS for the detection of pesticide residues on plant surfaces. Tricyclazole was first sprayed on the surfaces of three kinds of metal disks and detected using four moving mirror velocities. After parameter selection and optimization, pesticide residues were then detected on the surfaces of fresh rice leaves and ripe husks. Considering the varying thicknesses of plant cuticles and pesticide residues, depth-profiling FTIR-PAS has opened up new possibilities for optimally detecting pesticide residues on plant surfaces.

**RESULTS AND DISCUSSION**

**Spectral Investigation of Tricyclazole.** The molecular structure of tricyclazole (Figure 1) consists of a five-membered N-heterocyclic ring containing three nitrogen atoms and two double bonds separated by a single bond, thus constituting a \(\pi-\pi\) conjugated system. Figure 1 shows the spectra of the standard sample of tricyclazole scanned at four moving mirror velocities. The spectral photoacoustic signal (PA signal) of tricyclazole was higher at lower moving mirror velocities, whereas at higher moving mirror velocities such as 1.90 cm s\(^{-1}\), the PA signal was relatively unstable because of strong noise interference.24

Well-resolved peaks in the spectra could be successfully interpreted. A broad band between 2600 and 3400 cm\(^{-1}\) corresponded to the C–H stretching vibration.25 Two absorption peaks at around 1200 and 1000 cm\(^{-1}\) were assigned to the C–N stretching vibrations.26,27 A strong peak at approximately 1500 cm\(^{-1}\) resulted from aromatic ring vibrations overlapping with C\(=\)N vibrations.6,26,28 In the fingerprint region of IR, 1000–500 cm\(^{-1}\), a band occurring at around 750 cm\(^{-1}\) was assigned to C–S vibrations.25 The band around 1200 cm\(^{-1}\) emerged invariably and strongly at all four moving mirror velocities. Consequently, it could be regarded as the characteristic absorption peak for tricyclazole, thereby providing the basis for detecting organonitrogen pesticide residues.

**Spectra of Pesticide Residues on the Metal Surfaces.** The plant cuticle, which contains low amounts of nitrogen, is a hydrophobic layer with varied absorptivity to pesticides. In this study, metal surfaces of copper, aluminum, and iron were chosen to simulate the plant surfaces for photoacoustic spectroscopy, as they are similarly hydrophobic and do not contain nitrogen and differentially absorb pesticides.

Figure 2 shows the spectra around 1400–1000 cm\(^{-1}\) for the copper (Figure 2a,d), aluminum (Figure 2b,e), and iron (Figure 2c,f) disks sprayed with the tricyclazole sample (Figure 2a–c) and deionized water (Figure 2d–f). There were several absorption bands for copper disks sprayed with tricyclazole around 1000–1400 cm\(^{-1}\), including a strong band located at 1200 cm\(^{-1}\) and some relatively weaker bands located around 1300 and 1100 cm\(^{-1}\). The absorption bands of copper disks...
sprayed with deionized water appeared at the same wavenumbers, however, with a lower PA signal, especially around 1200 cm$^{-1}$. The absorption bands of aluminum disks sprayed with tricyclazole tended to be similar to those of copper in terms of their wavenumbers. The absorption bands at 1300 and 1100 cm$^{-1}$ sprayed with tricyclazole emerged to be similar to those sprayed with deionized water in terms of the PA signal, and similar results were obtained in the case of iron. We thus concluded that the bands at 1300 and 1100 cm$^{-1}$ should be assigned to the metal disks and some additives, respectively, whereas the band at 1200 cm$^{-1}$ corresponds to the C–N vibration from tricyclazole. These results establish the success of the FTIR-PAS method in detecting pesticide residues on various surfaces with different pesticide absorptivity.

**Optimization of Moving Mirror Velocity.** The photoacoustic spectrometer used in this study offers a series of moving mirror velocities to meet the requirements of different depth profiling. Table 1 shows the profiled depth under four moving mirror velocities at the range of 1500–400 cm$^{-1}$. Higher moving mirror velocities resulted in a thinner scanning depth and higher systematic noise. Because of the extremely thin pesticide residues (probably several micrometers) on the metal surfaces, selecting the most suitable moving mirror velocity was essential.

The spectra in Figure 2 indicate the characteristic absorption band emerging at 1200 cm$^{-1}$ at the moving mirror velocity of 0.95 cm s$^{-1}$, whereas the scanning depth was about 5.3 μm. Therefore, we can infer that the thickness of the pesticide sprayed on the metal disks was around 5.3–6.5 μm, which corresponds to the moving mirror velocity of 0.63 cm s$^{-1}$ at 1200 cm$^{-1}$.

The scanning depths at the lower moving mirror velocities of 0.63 and 0.32 cm s$^{-1}$ were deeper than 5.3 μm, resulting in the disappearance of the band around 1200 cm$^{-1}$. The scanning depth at 1.90 cm s$^{-1}$ was less than that at 0.95 cm s$^{-1}$ and should have theoretically resulted in the appearance of the typical tricyclazole band; however, the intense systematic noise resulting due to the very fast moving mirror velocity interfered with the target spectral signal. As a result, we concluded that 0.95 cm s$^{-1}$ was the most suitable moving mirror velocity for the detection of organonitrogen pesticide residues.

**Spectra of Pesticide Residues on the Rice Surfaces.** Figure 3 shows the spectra of fresh rice leaves (Figure 3a,c) and ripe husks (Figure 3b,d) sprayed with the pesticide sample (Figure 3a,b) and deionized water (Figure 3c,d) at 1400–1000 cm$^{-1}$. The higher moving mirror velocity of 1.90 cm s$^{-1}$ was excluded because of the intense systematic noise. The spectra of the rice surfaces sprayed with deionized water (Figure 3c,d) showed that there was a broad band at around 1100 cm$^{-1}$ in all three spectra obtained, which is usually attributed to the Si–O vibrations and C–O stretching modes. The spectra obtained at the three moving mirror velocities slightly differed from each other, and this could be explained by the inherent variations in the layered structure of the plant cuticle. A previous study on the characterization of tomato fruit cuticles by infrared spectroscopy has provided valuable insights about their chemical composition and structure; in fact, this layered structure abounds with cutin, wax, and polysaccharide and does not contain nitrogen.

The spectra of rice surfaces with tricyclazole residues (Figure 3a,b) demonstrated an obvious absorption peak at around 1200 cm$^{-1}$, scanned at a moving mirror velocity of 0.95 cm s$^{-1}$.

Table 1. Scanning Depths Using FTIR-PAS Technique at 400, 1200, and 1500 cm$^{-1}$ with Four Moving Mirror Velocities

| Wavenumber (cm$^{-1}$) | Scanning Depth$^{a}$ (μm) |
|-----------------------|---------------------------|
|                       | 0.32$^b$                  | 0.63$^b$                  | 0.95$^b$                  | 1.90$^b$                  |
| 400                   | 15.8                      | 11.2                      | 9.2                       | 6.5                       |
| 1200                  | 9.1                       | 6.5                       | 5.3                       | 3.7                       |
| 1500                  | 8.1                       | 5.8                       | 4.7                       | 3.3                       |

$^a$The values were calculated with the thermal diffusivity $D \approx 10^{-5}$ cm$^2$ s$^{-1}$.

$^b$Moving mirror velocities (cm s$^{-1}$) used in this study.

Figure 3. Spectra of fresh leaves (a,c) and ripe husks (b,d) of rice sprayed with the pesticide sample of tricyclazole (a,b) and deionized water (c,d) at a wavenumber range of 1400–1000 cm$^{-1}$. The scans were conducted in the midinfrared with a resolution of 4 cm$^{-1}$; the moving mirror velocities of 0.32, 0.63, and 0.95 cm s$^{-1}$ were involved for depth profiling of the samples; accumulating 256 scans per spectrum; with 10 repetition scans per sample at different positions to get an average spectrum; carbon black was used as the background.
which was different to the other two spectra scanned at 0.63 and 0.32 cm s\(^{-1}\) velocities. In contrast to the spectra of the rice surfaces sprayed with deionized water (Figure 3c,d), the bands of all three spectra around 1100 cm\(^{-1}\) were clearly ascribed to C–O and Si–O vibrations, but the visible absorption peak at 1200 cm s\(^{-1}\) with a moving mirror velocity of 0.95 cm s\(^{-1}\) corresponded to the C–N vibrations of tricyclazole (Figure 1). Noticeably, there was a slight absorption peak at 1200 cm s\(^{-1}\) at 0.63 cm s\(^{-1}\) in the spectrum of rice husk with tricyclazole residues (Figure 3b), and the PA signal was lower than the peak of the spectrum at 1200 cm s\(^{-1}\) at 0.95 cm s\(^{-1}\). One explanation for this observation could be that the absorptivity of ripe husk to tricyclazole was greater than that of fresh rice leaves, and thus, there was a greater amount of tricyclazole residue on the surfaces of the husks. Overall, the results indicate the success of FTIR-PAS in detecting tricyclazole residues on different rice surfaces as well.

**Principal Component Analysis.** Ten spectra of rice leaves and ripe husks sprayed with water (group 1) and tricyclazole (group 2) were recorded at the moving mirror velocity of 0.95 cm s\(^{-1}\). Principal component analysis (PCA) and the results showed that the first three principal components (PC1, PC2, and PC3) of the leaves (Figure 4a) and husks (Figure 4b)

Some samples were difficult to distinguish with PCA, probably owing to the uneven distribution of the pesticide or because the scores merely contained more information about the original independent variables but failed to establish any relationship between the independent and dependent variables.

**FTIR-PAS Scanning of the Plant Cuticle with Pesticide Residues.** As explained previously and depicted in Figure 5, the plant cuticle is a composite membrane with a heterogeneous spatial distribution, composed of an epicuticular wax layer, a cutin-rich fraction within intracuticular waxes, and phenolic compounds, and is connected to the cell wall by a polysaccharide-rich fraction with a thin layer of about 0.1–10 μm. As seen in Table 1, the scanning depth of the spectrometer at the moving mirror velocity of 0.95 cm s\(^{-1}\) attained 5.3 μm with optimized spectral recording parameters at 1200 cm\(^{-1}\), which is much smaller than 10 μm. In cases where there exist little or no pesticide residues, the scanning is likely to be performed directly on the cuticle layer, and the typical absorption would not appear at 1200 cm\(^{-1}\). Considering the thickness of the pesticide residues and cuticle layers, as shown in Figure 5, it is possible to optimize the scanning depth according to the specific plant cuticle, to ensure that the scanning remains in the pesticide layer, whilst maintaining a high signal-to-noise ratio.

**MATERIALS AND METHODS**

**Pesticide and Metal Substrates.** A wettable powder of tricyclazole with an active ingredient of 75% (Yifan Co., China) was selected as the pesticide and dispersed in distilled water at a ratio of 1:300 (w/v), which corresponded to the typical value in agricultural applications. The spectra of the standard tricyclazole sample (Aladdin Co., USA) served as the reference data.

Three disks made of copper, aluminum, and iron (diameter 10 mm and thickness 2 mm) were selected as the metal
substrates. These disks were first washed with dilute hydrochloric acid and distilled water, dried, and then classified into two groups. One group was sprayed with the tricyclazole suspension, whereas the other served as a control group and was sprayed with deionized water. To ensure homogenous spraying on the surfaces, both the suspension and water were sprayed thrice and through the same atomizer. All disks were then kept for drying at room temperature and at a relative humidity of 30% for 24 h.

**Rice Leaves and Husk Samples.** Fresh rice leaves and unhusked rice were randomly selected and divided into two respective groups each. Similar to the metal disks, the leaves were cut into disks with a diameter of 10 mm, and the treatment was repeated exactly as described for the metal disks described above.

**Spectra Recording.** Photoacoustic spectra for all samples were recorded using a Fourier transform infrared spectrometer (Nicolet 6700, Thermo Scientific, USA) equipped with a photoacoustic cell (model 300, MTEC, USA). The sample was placed in the cell holding cup (diameter 10 mm, height 3 mm), the cell was purged dry using dry helium (5 mL min\(^{-1}\)) for 10 s to ensure a CO\(_2\) and H\(_2\)O-free environment. The scans were performed in the midinfrared wavenumber range of 4000–500 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\), using four moving mirror velocities of 0.32, 0.63, 0.95, and 1.90 cm s\(^{-1}\) for depth profiling of the samples. Two hundred fifty-six scans were accumulated per spectrum, with 10 repetitions per sample at different positions to get an average spectrum, and the obtained spectra were normalized against a carbon black reference.

**Data Processing.** All obtained spectra were filtered using the Savitzky–Golay function (25 points and first polynomial order filtering) and standardized by the software MATLAB 2016b (MathWorks Inc., USA). Ten replicates for each sample were averaged by the mean function of MATLAB 2016b.

The average profiling depths of specific absorption bands under different modulation frequencies were calculated using the following function:

\[
\mu = \sqrt{\frac{D}{f}}
\]

where \(\mu\) denotes the thermal diffusion length, \(D\) denotes the leaf thermal diffusivity of the leaf, \(D \approx 10^{-3} \text{ cm}^2 \text{s}^{-1}\) for the polymer materials, and \(f\) denotes the modulation frequency (Hz), which equals wavenumber plus moving mirror velocity.

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**Notes**
The authors declare no competing financial interest.

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