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# Residue Dynamic of Pyrimorph on Tomatoes, Cucumbers and Soil under Greenhouse Trails

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**Abstract** A modified QuEChERS method for analysis of pyrimorph residue in tomatoes, cucumbers and soil was developed and validated. Residue dynamics and final residues in greenhouse vegetables and soil were studied. At fortification levels of 0.05, 0.1 and 1 mg kg$^{-1}$ in tomatoes, cucumbers and soil, the method got recoveries ranged from 86.1% to 99.3% with relative standard deviations of 1.0%–7.7%, in agreement with directives for method validation in residue analysis. The limit of determination in tomatoes, cucumbers and soil was 0.05 mg kg$^{-1}$. The proposed method was successfully employed for the determination of pyrimorph residue levels and dissipation rates in vegetables and soil. At six experimental sites, pyrimorph residues in tomatoes and cucumbers showed relatively fast dissipation rates, with half-lives of 5.8–7.7 days and 5.7–7.1 days respectively. Half-lives of pyrimorph in soil were 8.5–11.0 days. The final residues of pyrimorph in tomatoes ranged from 0.19 to 3.66 mg kg$^{-1}$, 0.18 to 4.35 mg kg$^{-1}$ in cucumbers and 0.22 to 16.5 mg kg$^{-1}$ in soil with pre-harvest interval of 3–7 days. 5 mg kg$^{-1}$ was proposed as the MRL of pyrimorph in tomatoes and cucumbers.

**Keywords** Pyrimorph · Residue dynamics · Greenhouse

Tomatoes and cucumbers are nutritious and tasty vegetables and popular with many people. The tomato late blight and the cucumber downy mildew can do serious harm to tomatoes and cucumbers. At present, the prevention and cure of these diseases mostly depend on chemical pesticides.

Pyrimorph, (E)-3-[(2-chloropyridine-4-yl)-3-(4-tert-butylphenyl)-acryloyl] morpholine, is a novel fungicide developed by China Agricultural University (Mu et al. 2007; Qin et al. 2005). Pyrimorph showed good activities in the efficacy test with GAP application method developed.

Currently maximum residue limit (MRL) in tomatoes and cucumbers for pyrimorph in China and other countries was not established. The objective of this study is to obtain the adequate residual data of pyrimorph under supervised trials in greenhouse tomatoes and cucumbers. Up to now no reports have been published on the dissipation and final residues of pyrimorph in tomatoes, cucumbers and soil at GAP application.

Developing and validating analytical methods of target compounds (may vary according to pesticide residue definition) is precondition for conducting supervised residual study. A quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction procedure has been developed recently for the extraction of pesticides from fruits and vegetables (Lehotay et al. 2005; Wang et al. 2007; Lesueur et al. 2008). The QuEChERS approach minimized the number of sample-preparation steps and provided good recoveries for a large number of pesticides. With the deepening of research on this method, QuEChERS has been applied to the sample preparation of grain (Nguyen et al. 2008), olive oil (Cunha et al. 2007), soil (Lesueur et al. 2008a) and other matrixes.

The present study was designed to investigate residue dynamics and final residues in greenhouse tomatoes, cucumbers and soil under supervised trails including application dosage, application frequency and pre-harvest

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interval (PHI). Methods which were based on QuEChERS methodology had been developed in the sample preparation of tomatoes, cucumbers and soil. Experiments were conducted to ensure the scientific application of 20% pyrimorph suspension in tomatoes and cucumbers and contributed the residue data to the risk assessment which addressed important decision on the MRL enforcement.

## Materials and Methods

Pyrimorph (purity, ≥99.5%) was from synthesis laboratory of China Agricultural University. The chemical structure of pyrimorph was shown in Fig. 1. HPLC-grade methanol and acetonitrile were purchased from SK Chemicals Co. Ltd (Korea). Deionized water was obtained from the Milli-Q SP Reagent Water system (Millipore, Bedford, MA, USA). Anhydrous $MgSO_4$ and NaAc, which were analytical grade, were obtained from Beijing Chemical and Reagent Ltd., Beijing, China. Primary secondary amine (PSA), was purchased from Agilent Technologies (Calif., USA).

The QuEChERS method was used as follows with slight modifications: (1) weigh 15 g of thoroughly homogenized sample into a 50 mL Teflon centrifuge tube; (2) add 15 mL MeCN (add 9 mL water in advance for the soil samples) then vortex for 1 min; (3) add 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc, shake vigorously by hand until well-mixed, after that extracting fully for 1 min using the vortex mixer; (4) centrifuge the tube at 4000 rpm for 5 min; (5) transfer 1 mL extract to a 2 mL plastic centrifuge tube containing 50 mg PSA (for cucumbers, 70 mg PSA) and 150 mg anhydrous $MgSO_4$; (6) mix the extract with the sorbent/dessicant for some time; (7) centrifuge the tube at 8,000 rpm for 5 min; (8) filtrate and transfer the acetonitrile layer into the vial for analysis.

The analysis of pyrimorph residues in tomatoes and cucumbers was performed on HPLC with diode array detector, low pressure quaternary pump (Agilent 1100, USA). Separations were carried out using a Shim-pack VP-ODS column (150 mm × 4.6 mm) from GL-Science, Japan, with 5 μm particle size. The mobile phase, which was composed of methanol: acetonitrile: water = 50: 25: 25 (v:v:v), was pumped at a flow rate of 1.0 mL $min^{-1}$. The column temperature was 25°C and the injection volume was 20 μl using the autosampler. The detection wavelength was 243 nm. The analysis of pyrimorph residues in soil was performed on HPLC with diode array detector, binary pump (Shimadzu LC-20AT, SPD-M20A detector, Japan). The chromatographic conditions were identical with the depiction above except for the mobile phase, which was composed of methanol: water = 75:25 (v:v).

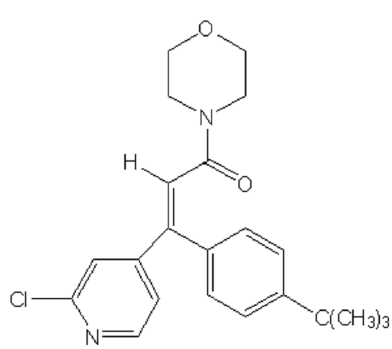


**Fig. 1** Chemical structure of pyrimorph

The study included the final residue experiment and the residue dynamic experiment was conducted at the greenhouses in Beijing, Jiangsu and Hunan during 2008–2009. There were four test areas at each experimental plot. The scheme was detailed shown in Table 1.

There were two application dosages in the final residual experiment and two application frequency were selected for each dosage. There were four tests in all, with three replications for each one. Twelve plots were needed and the area of each plot was 15 $m^2$. The stems and leaves of tomatoes and cucumbers were sprayed evenly with 2,250 or 4,500 g 20% pyrimorph suspension mixed with 900 kg water. For dynamic experiments spraying was done only one time. Three plots were needed and the area of each plot was 15 $m^2$. There was a vacant land offered to conduct the dynamic experiment for soil.

Treated samples were localized and tagged. Samples were collected at random from sampling plots, ensuring the samples were reliable and representative. The sampling amount of vegetables was required all of 2 kg. The sampling of soil was done on the surface layer of the plantation, with the deepness of 0–15 cm. The amount of soil samples was usually all of 1 kg. Sampling dates were shown in Table 1. Samples were packed with polyethylene bags and transported to the laboratory where they were chopped and thoroughly mixed. The sub-samples were kept at −20°C until analysis.

## Results and Discussion

In the classical QuEChERS method, PSA and anhydrous $MgSO_4$ were adding in extract and then vortex for 1min usually. In this research HPLC–DAD was selected as the analytical instrument and components of tomatoes and cucumbers were very complex. Only vortex for 1 min cannot meet the need of this research for purification. We selected oscillating in order to make adsorbing purification of PSA fully. In preliminary test we found that purification effect was related to standing time for the mixture of PSA, anhydrous $MgSO_4$ and sample extract. Purifying fully needed 5 h at least only by standing. By oscillating satisfying purification effect was gotten for both 2 h and 3 h. Finally the

**Table 1** Supervised greenhouse trails of 20% pyrimorph suspension in tomatoes and cucumbers

| Test type | Application rates (ai. g $ha^{-1}$) | Spray times/ frequency (d) | Pre-harvest intervals (d) | | | Samples |
|---|---|---|---|---|---|---|
| Final residue | 2,250 | 2 (6) | 3 | 5 | 7 | Tomatoes, cucumbers and soil |
| | | 3 (6/7) | 3 | 5 | 7 | |
| | 4,500 | 2 (6) | 3 | 5 | 7 | |
| | | 3 (6/7) | 3 | 5 | 7 | |
| Residue dynamics (tomatoes, cucumbers and soil) | 4,500 | 1 | 0, 1, 3, 5, 7, 9, 14, 21, 30 | | | Tomatoes, cucumbers and soil |
| Control | – | – | 0, 30 | | | Tomatoes, cucumbers and soil |

**Table 2** Results of residue dynamics of tomatoes and cucumbers

| Test site | Year | Tomato | | | Cucumber | | |
|---|---|---|---|---|---|---|---|
| | | Dissipation equation | $R^2$ | Half-life/d | Dissipation equation | $R^2$ | Half-life/d |
| Beijing | 2008 | $C = 1.9049e^{-0.1201x}$ | 0.971 | 5.8 | $C = 2.8646e^{-0.102x}$ | 0.9733 | 6.8 |
| | 2009 | $C = 2.3856e^{-0.1004x}$ | 0.9787 | 6.9 | $C = 1.8588e^{-0.113x}$ | 0.9618 | 6.1 |
| Jiangsu | 2008 | $C = 1.9099e^{-0.0988x}$ | 0.9668 | 7.0 | $C = 1.3732e^{-0.1032x}$ | 0.9727 | 6.7 |
| | 2009 | $C = 1.2742e^{-0.1092x}$ | 0.9716 | 6.3 | $C = 3.6069e^{-0.1226x}$ | 0.9627 | 5.7 |
| Hunan | 2008 | $C = 1.3693e^{-0.1085x}$ | 0.9534 | 6.4 | $C = 2.5494e^{-0.1219x}$ | 0.9819 | 5.7 |
| | 2009 | $C = 2.7655e^{-0.0904x}$ | 0.9573 | 7.7 | $C = 1.8313e^{-0.0977x}$ | 0.9647 | 7.1 |

purification process which we selected was as follows: transfer 1 mL extract to a 2 mL plastic centrifuge tube containing 50 mg PSA (for cucumbers, 70 mg PSA) and 150 mg anhydrous $MgSO_4$; vortex for 1 min and then oscillate for 2 h with moderate speed at 25°C. Less interference components in soil were present according to the blank chromatogram. The purification process was as follows: transfer 1 mL extract to a 2 mL plastic centrifuge tube containing 50 mg PSA and 150 mg anhydrous $MgSO_4$; vortex for 2 min.

Different concentrations of pyrimorph standard solutions (0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 mg $L^{-1}$) were prepared by diluting the stock solution in acetonitrile. Analyze and determine all the standard solutions under the same instrumental and chromatographic conditions described in 2.3. The injection volume was 20 μL. Quantification was accomplished by using the calibration curve constructed by the absolute amounts of pyrimorph against peak areas. A good linearity was achieved between the range 1–100 ng. The calibration curves were $y = 2.4031x+0.0204$ ($R^2 = 1$) under Agilent 1100 chromatographic condition and $y = 2844.8x+54.826$ ($R^2 = 0.9999$) under Shimadzu LC-20AT chromatographic condition.

Some standard solutions were fortified in tomatoes, cucumbers and soil without any pesticides. Fortified concentrations were 0.05 mg $kg^{-1}$, 0.1 mg $kg^{-1}$and 1.0 mg $kg^{-1}$ for each matrix. Five parallel samples were extracted, purified and analyzed according to above methods for each fortified concentration. The results showed that average fortified recoveries of pyrimorph were 95.4%–99.3% for tomatoes with RSD of 2.3%–7.7%, 95.2%–98.7% for cucumbers with RSD of 1.0%–3.4%, 86.1%–96.2% for soil with RSD of 2.0%–5.6%. The limit of determination in tomatoes, cucumbers and soil was 0.05 mg $kg^{-1}$. The addition recovery test demonstrated that the recovery and precision of the methods satisfied the standards of pesticide residue analysis.

Pyrimorph residue reached its peak one day after spraying to tomatoes, cucumbers and soil once at 4,500 ai. g $ha^{-1}$. The residues of 6 experimental sites were 1.32–2.81 mg $kg^{-1}$ for tomatoes, 1.43–3.73 mg $kg^{-1}$ for cucumbers and 2.14–4.98 mg $kg^{-1}$for soil. In tomatoes and cucumbers, the rate of pyrimorph dissipation is relatively fast. The half-lives for tomatoes were 5.8–7.7 days and 5.7–7.1 days for cucumbers (Table 2). The rate of

**Table 3** Results of residue dynamics of soil

| Test site | Year | Soil | | |
|---|---|---|---|---|
| | | Dissipation equation | $R^2$ | Half-life/d |
| Beijing | 2008 | $C = 1.9014e^{-0.0629x}$ | 0.9581 | 11.0 |
| | 2009 | $C = 4.3931e^{-0.0721x}$ | 0.959 | 9.6 |
| Jiangsu | 2008 | $C = 2.2783e^{-0.0819x}$ | 0.9688 | 8.5 |
| | 2009 | $C = 2.5523e^{-0.0658x}$ | 0.9448 | 10.5 |
| Hunan | 2008 | $C = 4.1823e^{-0.069x}$ | 0.9649 | 10.0 |
| | 2009 | $C = 4.5989e^{-0.0777x}$ | 0.9336 | 8.9 |

**Table 4** Results of pyrimorph final residues on tomatoes, cucumbers and soil under GAP application

| Application rates (ai. g $ha^{-1}$) | Spray times | Pre-harvest intervals (d) | Final residue (mg $kg^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | Tomato | Cucumber | Soil*[a] | Soil*[b] |
| 2,250 | 2 | 3 | 0.28–1.35 | 0.57–2.38 | 0.63–1.98 | 0.64–1.85 |
| | | 5 | 0.27–1.00 | 0.36–1.51 | 0.55–0.75 | 0.38–1.29 |
| | | 7 | 0.19–0.99 | 0.18–0.85 | 0.45–1.04 | 0.26–0.66 |
| 2,250 | 3 | 3 | 0.56–1.82 | 1.14–2.66 | 0.96–3.78 | 0.80–5.40 |
| | | 5 | 0.41–1.52 | 0.81–1.62 | 0.48–3.52 | 0.22–2.27 |
| | | 7 | 0.26–1.25 | 0.51–1.90 | 0.45–3.18 | 0.69–2.19 |
| 4,500 | 2 | 3 | 0.78–2.14 | 2.25–2.96 | 1.66–6.00 | 2.72–10.84 |
| | | 5 | 0.54–2.47 | 1.33–2.73 | 0.98–3.42 | 2.11–7.16 |
| | | 7 | 0.55–2.03 | 0.56–1.71 | 0.70–3.21 | 1.51–4.24 |
| 4, 500 | 3 | 3 | 1.74–3.66 | 2.62–4.35 | 5.61–16.47 | 6.44–9.87 |
| | | 5 | 1.21–3.05 | 1.85–3.06 | 1.18–11.77 | 5.60–9.82 |
| | | 7 | 0.65–1.96 | 1.09–2.79 | 0.72–5.37 | 2.14–5.37 |

**Table 5** Results of pyrimorph final residues PHI of 5 days on tomatoes and cucumbers under GAP application

| | Final residues (mg $kg^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tomato | 0.27 | 0.32 | 0.41 | 0.42 | 0.45 | 0.48 | 0.54 | 0.68 | 0.77 | 0.97 | 0.97 | 1.00 |
| | 1.21 | 1.26 | 1.26 | 1.27 | 1.30 | 1.32 | 1.48 | 1.52 | 1.91 | 1.96 | 2.47 | 3.05 |
| Cucumber | 0.36 | 0.39 | 0.58 | 0.76 | 0.81 | 0.90 | 0.90 | 1.13 | 1.33 | 1.41 | 1.51 | 1.53 |
| | 1.58 | 1.62 | 1.64 | 1.85 | 1.86 | 2.29 | 2.33 | 2.62 | 2.73 | 2.77 | 3.00 | 3.06 |

pyrimorph dissipation is slow in soil. The half-lives were 8.5–11.0 days (Table 3).

Results of the final residue experiment were shown in Table 4. All the residues in tomatoes, cucumbers and soil were proportional to the application dosage and the application frequency, inversely proportional to pre-harvest intervals.

Maximum Residue Limit (MRL) in tomatoes and cucumbers of pyrimorph was not established in China. The oral no observed adverse effect level of pyrimorph in SD male and female rats were 30 mg kg $bw^{-1}$ $d^{-1}$ according to studies on the toxicity. Proposed acceptable daily intake (ADI) of pyrimorph was 0.3 mg kg $bw^{-1}$ $d^{-1}$ assuming that the coefficient of safety was 100. According to the results, the residues in tomatoes were less than 3.66 mg $kg^{-1}$ at pre-harvest interval 3 days and less than 3.05 mg $kg^{-1}$ at pre-harvest interval 5 days. Those in cucumbers were 4.35 mg $kg^{-1}$ and 3.06 mg $kg^{-1}$ respectively. 5 mg $kg^{-1}$ was suggested as the proposed MRL of pyrimorph in tomatoes and cucumbers.

The STMRs of tomatoes and cucumbers were 1.11 mg $kg^{-1}$ and 1.56 mg $kg^{-1}$ respectively according to Table 5. The IEDIs were calculated with Formula.1, where 60 (kg) was the standard body weight of human beings and 0.5 (kg) was the daily dietary intake of vegetables according to Chinese Dietary Guide (2007) revised by Ministry of Health.

$$\mathrm{IEDI} = \mathrm{STMR} \times 0.5/60 \tag{1}$$

The IEDIs of tomatoes and cucumbers were 0.009 mg kg $bw^{-1}$ $d^{-1}$ and 0.013 mg kg $bw^{-1}$ $d^{-1}$ respectively. Both of them were far less than ADI of pyrimorph. The risk assessment of short term intake was not significative because of the low toxicity of pyrimorph. The recommended MRL (5 mg $kg^{-1}$) is acceptable under current recommend GAP.

Applying 20% pyrimorph suspension to control tomato late blight and cucumber downy mildew by spraying 1–2 times at 2,250 ai. g $ha^{-1}$, PHI of 5 days on tomatoes and cucumbers was recommended based on the final residue test and the above single-point risk assessment results.

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