Comparison of Analytical Methods and Residue Patterns of Pymetrozine in *Aster scaber*

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Abstract Residues of the polar pesticide pymetrozine were compared using two methods: hydromatrix and liquid-liquid extraction (LLE). The biological half-life and the final residue level were investigated using *Aster scaber* over a 10-days cultivation period. The respective biological half-lives of the pesticide were 4.2 and 3.5 days at the recommended and double dose. The final residue levels were 1.28 and 1.98 mg kg\(^{-1}\), respectively, at the same application rate of pymetrozine according to the GAP standard of the United Kingdom. Average recovery was higher with LLE than with the hydromatrix method. Dissipation curves of pymetrozine were influenced by the application amount and growth rate of *A. scaber*. The final residue level of pymetrozine could be predicted to be lower than the UK maximum residue limit for lettuce applying the GAP standard.

Keywords Pymetrozine · LLE · Hydromatrix · Growth rate

Pymetrozine [(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one] (Fig. 1) is a novel selective insecticide that represents a new chemical class of insecticides with remarkable selectivity for plant-sucking insects such as aphids (Fuog et al. 1998; Cun et al. 2011). It acts in a unique way by interfering in the regulation of the nervous system for feeding behavior, which results in death of the insect due to starvation a few days after application (Bextine et al. 2004; Guoqing et al. 2009). The US Environmental Protection Agency (EPA) has classified pymetrozine as a “likely” human carcinogen, and the data are insufficient to eliminate the need for a quantitative risk assessment. Because of the limited number of sites on which this pesticide is used, the low use rates, and low exposure, the actual risks to human health are below the level of concern (EPA 2000).

*Aster scaber* is an edible dicotyledonous plant in the family Asteraceae (Compositae), the same family as lettuce. The maximum residue limit (MRL) for pymetrozine on lettuce has been established in the United Kingdom at 2 mg kg\(^{-1}\) (HSE 2008). Several methods have been developed for analyzing pymetrozine residues in plants, such as an enzyme immune assay (ELA), liquid chromatography (LC) with UV/vis, diode array detection (DAD), and mass spectrometry (MS). However, the most frequently used method is LC with UV/vis DAD (Peter et al. 1996; Zhang et al. 2007). Polar pesticides like pymetrozine (log Kow, −0.18) and dinotefuran (log Kow, −0.549) are known to be difficult to analyze. Therefore, this study sought to reveal efficient analytical methods for detecting pymetrozine residues and examined the biological half-life and residue pattern of this pesticide in *A. scaber* during cultivation. At the same time, the final residue levels at harvest for *A. scaber* and lettuce were predicted after pymetrozine was sprayed according to the GAP standard of the United Kingdom.

Materials and Methods

A formulation containing 25% pymetrozine WP (CHESS WP®) was purchased from Syngenta Korea Inc., Republic of Korea. The reference standard for pymetrozine (purity, 99.9%) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Methanol, dichloromethane, ethyl acetate, and
Liquid–liquid Extraction (LLE) Method

An A. scaber sample (10 g) was extracted with 60 mL methanol and 7 mL 0.05 M borax buffer, and filtered under vacuum through a Buchner funnel. The container and filter cakes were washed with 30 mL methanol, and the extracts were combined. The sample was evaporated in a vacuum rotary evaporator (EYELA, Japan) at 40°C until the extract volume reached about 10 mL, then 3 mL of saturated sodium chloride was added. The sample was evaporated to dryness on a vacuum rotary evaporator at 40°C, and the residue was dissolved in 2 mL methanol/water (2:8, v/v) for analysis by HPLC.

Results and Discussion

An external standard method was adopted in this experiment. A six-point calibration curve from 0.025 to 5.0 mg kg−1 (peak area vs. concentration) was constructed for quantitative analysis of pymetrozine. The linear equation was \( y = 130716x + 952.92 \) (\( R^2 = 1.0000 \)).

Pymetrozine was added to untreated control samples at two levels (0.5 and 2.5 mg kg−1). For method validation, control and fortified samples were analyzed under the same conditions. The reported results are mean of three replicates, whereby the coefficient of variation (CV) was within 10%. The results of the recovery studies are presented in Table 1. To compare chromatograms between LLE and hydromatrix, HPLC chromatograms are provided in Fig. 2. The limit of quantitation (LOQ) of pymetrozine in

Table 1 Comparison of recovery methods for pymetrozine from Aster scaber (\( n = 3 \))

| Fortified level (mg kg−1) | Mean recovery (±CV) (%) |
|--------------------------|-------------------------|
|                          | LLE                     | Hydromatrix               |
| 0.5                      | 97.2 ± 2.13              | 81.6 ± 8.69               |
| 2.5                      | 86.3 ± 3.02              | 69.9 ± 4.76               |
A. scaber was calculated as 0.05 mg kg\(^{-1}\) and the minimum detectable quantity was 5 \times 10^{-10} g. Average recovery was higher with the LLE than the hydromatrix method, and the CV was more precise for the former.

Plant residue estimations for pymetrozine were carried out over 10 days following the spraying treatment. Initial (2 h) residues were 2.86 and 6.21 mg kg\(^{-1}\) at the recommended dose and double dose, respectively. Ten days after application, the remaining residues of pymetrozine were 0.60 and 0.79 mg kg\(^{-1}\); i.e., 79.02% and 87.28% of the initial residue levels had already dissipated. Figure 3 shows the change in pymetrozine residues for the seven sample times at 0–10 days after spraying. The kinetics of pymetrozine can be described with the following equations, 

\[
y = 2.8706e^{-0.1644t} \quad (R^2 = 0.9799) \quad \text{and} \quad y = 5.9791e^{-0.1996t} \quad (R^2 = 0.9950),\]

and the biological half-life in A. scaber was 4.22 and 3.47 days at the recommended dose and the double dose, respectively.

The average weight of representative plant samples after 0–10 days were 1.87 ± 0.23 g (n = 20) at day 0 and increased to 4.78 ± 0.41 g (n = 20) at day 10. However, the average weight of representative A. scaber cultivated under the vinyl house differed according to cultivation date. Over 10 days, plant weight increased 2.56-fold from 17–27 May 2011 and 5.45-fold from 29 April to 9 May 2011 (NAQS 2011).

The pesticide dilution effect (f\(_0\)) was calculated from the remaining pymetrozine and weight of A. scaber over 10 days (Kim et al. 2009). For the three cases of considered growth rate, non-considered growth rate (NGR), 2.56-fold growth rate (Low Growth Rate, LGR), and 5.45-fold growth rate (High Growth Rate, HGR), the residue pattern in relation to growth at the double dose are given in Fig. 4. The biological half-life was 7.55, 3.47, and 1.91 days in the case of NGR (y = 5.3410e^{-0.0919t}), LGR (y = 5.9791e^{-0.1996t}), and HGR (y = 6.8575e^{-0.3636t}), respectively. Therefore, the growth rate of A. scaber influences the residue levels of pymetrozine.

\[
f'_0 = \frac{\text{Residue at 0 days} \times \text{Weight of A. scaber at 0 days}}{\text{Weight of A. scaber at sample collection}}\]

The rapid growth rate of cucumber will cause a dilution of pesticide residues over time, even if the pesticide does not degrade (Metwally et al. 1997). Moreover, Talebi and
Ahsaii (2006) reported that no residues were detected in cucumber 4 days after spraying with pymetrozine under field conditions.

The final residue levels in A. scaber were compared when pymetrozine was sprayed according to the GAP standard of the United Kingdom for lettuce (application: three times at a spray interval of 7 days, and a maximum dose of 0.4 kg a.i. ha\(^{-1}\), PHI: 7 days). The prediction was calculated based on the initial amount sprayed and the coefficient of regression (Kim et al. 2009). Pymetrozine residues according to application amount are given in Fig. 5. After spraying with 1.2 kg ha\(^{-1}\) (double dose, 0.4 kg ha\(^{-1}\) × 3), the final residue level was predicted to be 1.98 mg kg\(^{-1}\) at harvest. After spraying with 0.6 kg ha\(^{-1}\) (recommended dose, 0.2 kg ha\(^{-1}\) × 3), the residue level was 1.28 mg kg\(^{-1}\). From this prediction, the pymetrozine residue on lettuce was below the maximum residue limit of the United Kingdom (2 mg kg\(^{-1}\)) 7 and 5 days after spraying with a double dose or the recommended dose, respectively.

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