

DISSIPATION AND TRANSFORMATION OF INSECTICIDE RESIDUES IN FRESH, DRIED ROSES AND TEA (ROSE INFUSION)

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Abstract: Modified QuEChERS method was applied to study the dissipation of insecticides in rose and its processing products. Concentrated rates of pesticide residues were 1.5-5.3 times from fresh roses transferred to dried ones at 80 °C for 3 h. Approximately 1.1-86.9% of residues were soaked to the infusion from dried roses by boiling water. The rapid method allowed us to quickly inspect the residues of pesticides in rose processing products. The residues in rose flowers declined with time and reached below the Taiwan maximum residue limits (MRLs) of 0.5 mg/kg for chlorpyrifos and imidacloprid.

Keywords – Rose; Dissipation; Processing; Insecticide residues

I. INTRODUCTION

The rose flowers into dish and tea have been popular and reported to have some efficacies for health and alternative therapy [1]. Rose is one of the most important imported and domestic flowers around the world. High temperature contributes to spread of pests so quickly that insecticides are necessary for pest control. The integrated pest management strategies require spraying insecticides with different modes of action during the cultivation of roses. The pesticide residues in flowers and their processing products have been noticed and become the public concern. Procymidone and pyrimethanil detected (0.16-0.70 mg/L, below the EU MRL in wine grapes) in three of 20 rose wine samples was reported in Spain [2]. Azoxystrobin (0.25 µg/L) and carbofuran (0.24-11.1 µg/L) was detected in three of 4 rose wine samples in Brazil [3]. Some herbal samples (chamomile, marjoram, and thyme) from Egyptian markets contaminated by pesticides but most of residues were not exceeded the EU permissible limits [4]. The GC-MS/MS method for the pesticide determination in samples of rose concrete and absolute was developed [5], and chlorpyrifos (18.9-27.3 mg/kg) detected in rose concrete and absolute samples was investigated and reported in France [6].

A rapid method to determine the residues of three different family pesticides in fresh, dried roses and tea (rose infusions) by modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method with GC-MS/MS and LC-MS/MS was developed and applied to study the dissipation and transformation of residues in rose samples after pesticide applications in trials. Three pesticides, chlorpyrifos, methomyl, and imidacloprid, with significantly different physicochemical properties (Table 1) were selected to test.

TABLE I
 SUMMARIZE OF PHYSICOCHEMICAL PROPERTIES OF THREE INSECTICIDES

	chlorpyrifos	methomyl	imidacloprid
Family	organophosphate	carbamate	neonicotinoid
Mol.wt.	350.6	162.2	255.7
Solubility in water (mg/L, 20-25°C)	1.05	57900	610
Stability	DT ₅₀ 1.5 d (pH 8) to 100 d (pH 7), can form chelates with metals	stable in water for 30 d (pH 5 and 7, 25°C), stable up to 140°C	stable to hydrolysis at pH 5-11
Mode of action	non-systemic, contact, stomach, respiratory	systemic, contact, stomach	systemic, contact, ingestion
IRAC classification ^a	1B	1A	4A

^aThe "Mode of Action Classification" fifth ed. Electronic file updated April 2016, The Insecticide Resistance Action Committee (IRAC).

II. MATERIALS AND METHODS

Chemicals and Solvents: All of the solvents were pesticide grade (TEDIA, USA) or analytical grade and purchased from Merck Co. (Darmstadt, Germany). Magnesium sulfate anhydrous (98%, Scharlau), ammonium acetate (analytical grade) and sodium acetate trihydrate (99.5%, Sigma-aldrich) was purchased from Merck (Taipei,

Taiwan). Primary secondary amine (PSA) was purchased from Agilent (Taipei, Taiwan). Reference standards (>95%) of chlorpyrifos, methomyl, and imidacloprid were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solutions were prepared with acetone or acetonitrile in a concentration of 500 or 1000 µg/mL. Working solutions were prepared by dilution of the stock solution with acetone or acetonitrile. Stock solutions were diluted with proper solvent to prepare sample spiked standard solutions in the range of 0.01-0.2 mg/L of calibration curves. Commercial chlorpyrifos 40% EW, methomyl 24% SL, and imidacloprid 9.6% SL were purchased from Lianli Agricultural Technology and Huikwang Corporation, Taiwan.

Extraction and transformation of pesticides in rose flowers: To study the behaviour of pesticides during the transformation process of roses, fresh rose flowers collected from the fields were dried in the oven (80°C) for 3 h (Figure 1). Six gram of dried rose flowers was infused in 380 mL of boiling water. After 30 min of brewing, the water extract was filtered and cooled for the preparation of rose infusion samples. The modified QuEChERS method was used to extract three pesticide residues in rose flowers and infusions. Five grams of fresh rose flowers, two grams of dried rose powder (mixed with 5 mL of ultrapure water for 20 min), or 5 mL of rose infusion, respectively, was transferred into a 50 mL PTFE centrifuge tube and 20 mL of acetonitrile with 1% acetic acid was added. The centrifuge was shaken vigorously by vortex shaker for 1 min. Then 6 g of anhydrous MgSO₄ and 1.5 g NaCH₃COO were added and shaken by vortex for 1 min and cooled the tube in an ice water bath for 3 min followed by centrifugation at 6000 rpm (under 15°C) for 5 min. Six mL of upper layer was transferred to the dispersive tube which contained 150 mg PSA and 900 mg anhydrous MgSO₄. The dispersive tube was centrifuged at 6000 rpm condition described above. The extracted eluent was concentrated to dryness by evaporating with weak nitrogen stream at 40°C. The residue was reconstituted to 1 mL of solvent (acetonitrile with 1% acetic acid for LC-MS/MS and acetone for GC-MS/MS determination) and filtered with a 0.22 µm PVDF or PTFE filter for instrument analysis.

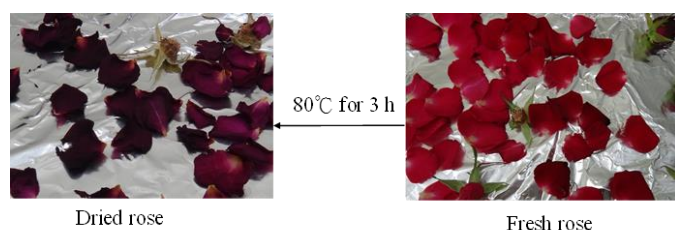


Fig. 1 Dry roses processing from fresh samples at 80°C oven for 3 h.

Apparatus: Liquid chromatography-tandem mass spectrometry (Waters XEVO-TQMS) with turbo ion spray source operating in positive ionization and using the multiple reaction monitoring (MRM) mode was used for quantification of methomyl and imidacloprid in samples. The following parameters were set: capillary voltage: 3.20 kV; source temperature: 150°C; desolvation temperature 320°C; cone gas flow: 100 L/h; desolvation flow: 600 L/h. To achieve the optimum sensitivity and resolution, the MRM

transition (163>88, 163>106 for methomyl qualitative analysis; 256>209, 256>175 for imidacloprid qualitative analysis), collision energy (10 and 20 eV) and cone voltage (20 and 28 V) were optimized for determination of methomyl and imidacloprid. Chromatographic separations were carried out on a Waters ACQUITY UPLC HSS T3 (1.8 µm, 100 × 2.1 mm i.d.) column. Isocratic analysis was performed with the gradient mobile phase of 10% methanol in 5 mM ammonium acetate (A) and 90 % methanol in 5 mM ammonium acetate (B) at a flow rate of 0.3 mL/min. The elution program started with 100% solution A at injection time, increase to 100% B by 5 min, was held at 100% B until 14 min.

Residue analyses of chlorpyrifos was carried out using Agilent 7890 A gas chromatography coupled with Waters Quattro micro MS/MS. The electron ionization mode at +70 eV with a sample injection volume of 1 µL. Chromatographic separations were carried out using DB-5 MS Ultra Inert column (30 m × 0.25 mm, 0.25 µm) which obtained from Agilent Technologies (Taiwan). A pure Helium gas (>99.999%) was used a carrier gas with a constant flow rate of 1 mL/min. The oven temperature program was initially held for 2 min at 90°C, 7°C/min to 300°C. The triple-quadrupole mass spectrometer was operated in the MRM mode. The MRM mode transition (314>258, 314>286 for qualitative analysis, collision energy of 12 and 6 eV) were optimized for determination of chlorpyrifos in samples.

Field Trials and Experimental design: The field experiments were conducted in rose (*Rosa spp.*) fields (Yunlin, Taiwan) in May and June, 2012. The experimental design was a split-plot arrangement of a randomized complete block with three replicates. The tank mix of chlorpyrifos 40% EW, methomyl 24% SL, and imidacloprid 9.6% SL was applied at the recommended dose (0.32, 0.27, and 0.03 kg a.i./ha) and the higher rates (0.45, 0.43, and 0.06 kg a.i./ha), respectively, with a knapsack power sprayer. The untreated control and treatments of three pesticides were conducted with one application at full flowering stage (BBCH principal growth stage 6, at least 50% of flowers open) of rose cultivation. Sample collection was initiated 3 h (for 0 day) after pesticide application. The process of collection was repeated at 3, 7, 11, 15, 18, and 21 days afterward to determine the residues of three pesticides in roses. Meteorological conditions were continuously recorded. During the period of pesticide application and sampling, daily total rainfall amounts ranged from 0 to 25.5 mm/day. The minimum and maximum of average temperatures were 25.5 and 29.5 °C. All collected samples in this study were stored frozen (-20 ± 5°C) for no longer than 100 days between sampling and instrument analysis. The stability of pesticides in rose flowers during frozen storage was evaluated. The study indicated that residues of three insecticides were stable in rose flowers under frozen condition.

III.RESULTS AND DISCUSSION

Validation of the method: The specificity of the analytical method was confirmed by analysis of blank rose flower and infusion samples in triplicate and by processing through the extraction procedures described above. The accuracy and precision of the analytical method were confirmed by the

recovery test of pesticides in rose samples. A good linear correlation between pesticide concentrations analyzed and the analytical response by the determination coefficients (R^2 : 0.995-0.999) of standard spiked calibration curves. The limits of quantification (LOQ) were ranged from 0.1 to 4.3 ppb ($\mu\text{g/L}$, $S/N=10$). The recoveries (Table II) ranged from 82 to 114% (fortification at 0.1, 0.4 and 0.8 $\mu\text{g/g}$ for fresh and dried rose samples; 0.02, 0.05 and 0.10 $\mu\text{g/mL}$ for rose tea, RSD: 0.9-9.4%, $n=3$).

TABLE III

MEAN RECOVERIES (R) AND RELATIVE STANDARD DEVIATIONS (RSD) OF CHLORPYRIFOS, METHOMYL, AND IMIDACLOPRID IN FRESH, DRIED ROSE FLOWERS, AND TEA (ROSE INFUSIONS)

Pesticides	% R (RSD) ^a		
	Fresh flowers	Dried flowers	Rose infusion s
chlorpyrifos	84-97 (5.4-9.4)	102-109 (1.2-6.1)	83-108 (1.1-5.7)
methomyl	84-92 (0.9-5.1)	86-95 (3.5-7.6)	102-114 (3.1-6.8)
imidacloprid	82-91 (1.4-5.4)	84-91 (1.4-8.5)	104-109 (2.7-8.9)

^a Fortification at 0.1, 0.4 and 0.8 $\mu\text{g/g}$ for fresh and dried rose samples; 0.02, 0.05 and 0.10 $\mu\text{g/mL}$ for rose tea, $n=3$.

Dissipation of pesticide residues in roses: The residues (Table III) of chlorpyrifos, methomyl, and imidacloprid in fresh roses declined from 68.0, 13.9, and 3.9 to 0.1, 0.2, and 0.2 $\mu\text{g/g}$, respectively, 11 days after application at the higher rates of 0.45, 0.43, and 0.06 kg a.i./ha. The residues in rose flowers declined with time and reached below the Taiwan maximum residue limits (MRLs) of 0.5 $\mu\text{g/g}$ for chlorpyrifos and imidacloprid. There is no tolerance standards for methomyl in rose in Taiwan. The persistence of imidacloprid has the 5.1% (much higher than the other two pesticides) of initial deposits in fresh roses on the 11nd day after pesticide application. This phenomenon is contributed to the much more stable and systemic property of imidacloprid. The structure of crop species and properties of pesticides significantly affects the residue levels [7]. The declined rate of pesticide residues on crops were different between Chinese cabbage (*Brassica pekinensis* Rupr.) and leafy kale vegetable (*Brassica alboglabra*) in fields according to our study [8]. We also found that the residues on head lettuce were higher than that on cabbage or broccoli vegetables after pesticide applications, but the dissipation rate of imidacloprid was slower than that of chlorpyrifos and methomyl in these vegetables [9].

Transformation of pesticide residues after rose processing:

The initial residue levels were 103.8, 32.0, and 9.6 $\mu\text{g/g}$ of chlorpyrifos, methomyl, and imidacloprid, respectively, in dried rose petals transferred from fresh samples collected at 0 days (3 h) after pesticide application at the higher rates of 0.45, 0.43, and 0.06 kg a.i./ha (Table IV). Concentrated rates of the three pesticides were 1.5-5.3 times transferred to dried roses from fresh samples under 80°C oven for 3 h. The processing factors (from fresh to dried rose flowers) of

chlorpyrifos, methomyl, and imidacloprid, were 1.5-1.7, 2.3-4.2, and 2.4-5.3, respectively. The un-stability (Table 1, low DT_{50} at pH 8) of chlorpyrifos resulted in the relative low transformation rates (1.5-1.7) during the 80°C processing condition. Approximately 1.1, 86.9, and 70.4% of chlorpyrifos, methomyl, and imidacloprid, respectively, were soaked to the tea samples from the dried roses by the boiling water. Residues of three pesticides in rose infusions (Table V) dissipated quickly after application and reached below the European Union MRLs of 0.5 (chlorpyrifos) and 0.05 (LOQ, methomyl and imidiclorpid) $\mu\text{g/g}$.

TABLE IIIII

RESIDUES OF CHLORPYRIFOS, METHOMYL, AND IMIDICLOPRID IN FRESH ROSE FLOWERS COLLECTED AFTER PESTICIDE APPLICATIONS IN TRIALS

Day	Residues ($\mu\text{g/g}$)					
	Chlorpyrifos		Methomyl		Imidicloprid	
	L	H	L	H	L	H
0	24.03	68.04	6.84	13.87	1.49	3.92
3	3.91	9.15	3.81	6.38	0.90	1.83
7	0.14	0.32	0.16	0.53	0.10	0.28
11	0.03	0.10	BDL	0.23	0.03	0.19
18	0.01	0.01	BDL	BDL	0.01	0.01
21	BDL	BDL	BDL	BDL	BDL	BDL

L, lower application rate (0.32, 0.27, and 0.03 kg a.i./ha); H, higher application rate (0.45, 0.43, and 0.06 kg a.i./ha); BDL, below the LOQs.

TABLE IVV

RESIDUES OF CHLORPYRIFOS, METHOMYL, AND IMIDICLOPRID IN DRIED ROSE FLOWERS TRANSFERRED FROM FRESH SAMPLES

Day	Residues ($\mu\text{g/g}$)					
	Chlorpyrifos		Methomyl		Imidicloprid	
	L	H	L	H	L	H
0	40.88	103.81	28.73	32.03	7.94	9.57
3	6.75	10.88	13.18	21.89	2.91	6.12
7	0.79	2.02	0.47	5.18	0.58	3.04
11	0.05	0.09	0.01	0.02	0.12	0.22
18	0.05	0.03	0.02	0.01	0.03	0.06
21	0.02	0.02	BDL	BDL	BDL	BDL

L, lower application rate (0.32, 0.27, and 0.03 kg a.i./ha); H, higher application rate (0.45, 0.43, and 0.06 kg a.i./ha); BDL, below the LOQs.

TABLE V

RESIDUES OF CHLORPYRIFOS, METHOMYL, AND IMIDICLOPRID IN ROSE INFUSIONS

Day	Residues ($\mu\text{g/g}$)					
	Chlorpyrifos		Methomyl		Imidicloprid	
	L	H	L	H	L	H
0	0.72	1.11	14.73	27.83	3.25	6.73
3	BDL	0.33	3.21	7.95	0.75	1.24
7	BDL	BDL	0.04	0.12	BDL	0.09
11	BDL	BDL	0.02	0.03	BDL	BDL
18	BDL	BDL	BDL	BDL	BDL	BDL
21	BDL	BDL	BDL	BDL	BDL	BDL

L, lower application rate (0.32, 0.27, and 0.03 kg a.i./ha); H, higher application rate (0.45, 0.43, and 0.06 kg a.i./ha); BDL, below the LOQs.

Physicochemical properties (water solubility, stability etc.) of pesticides significantly affects the transformation and dissipation of residues. The residues of lambda-chyalthrin (extremely low water solubility of 0.005 mg/L) in fresh green tea leaves and black tea were studied in India and the residues did not leach into the tea brew [10].

Specific physicochemical properties of three pesticides, chlorpyrifos, methomyl, and imidacloprid [11] summarized in Table I resulted in the different behaviours of residues in processing products. The lower transformation rate (1.1%) of chlorpyrifos from the dried rose flowers to the rose tea according to its non-systemic action and the lower water solubility of 1.05 mg/L. Methomyl and imidacloprid are both systemic insecticide with a relatively high water solubility of 57900 and 610 mg/L and stable to hydrolysis. These characters of the two insecticides resulted in the higher (70.4-86.9%) soaking process factors (rates) from dried rose petals to the infusions by boiling water. The similar phenomenon of pesticide residues during the crop processing were reported [6,12-13]. Percent transfer studies found that 5.7, 46.9 and 38.8% of chlorpyrifos residues from rose flowers was transferred to hydro-distillation rose water, concrete and absolute [12]. Typical pesticides were found to be concentrated by a factor of 100-300 from the rose flowers to the rose absolute [6]. The concentrations determined for the corresponding rose absolutes were 4.7 mg/kg for methidathion and 0.65-27.25 mg/kg for chlorpyrifos [6]. Residue levels of fenazaquin were determined in unprocessed and processed okra fruits to evaluate the effect of different processes (washing, boiling and washing followed by boiling) in reduction of this pesticide residues in okra. Maximum reduction (60-61%) was observed by washing + boiling followed by boiling/cook (38-40%) and then by washing (31-32%) of fenazaquin residues in okra fruits [13]. Specific physicochemical properties of pesticides and different processing procedures could significant effect the dissipation and transformation of residues.

IV. CONCLUSIONS

The residues of three insecticides belonging to different family (organophosphate, carbamate and neonicotinoid) were studied in fresh and dried rose flowers and its infusions after pesticide application. Modified QuEChERS methods were applied to study the dissipation of residues in rose samples after processing procedures. The residues in rose flowers declined with time and reached below the Taiwan MRLs of 0.5 mg/kg for chlorpyrifos and imidacloprid. Concentrated rates of methomyl and imidacloprid were 2.3-5.3 times from fresh roses transferred to dried ones at 80°C for 3 h. Higher percentage (70.4-86.9%) of residues of methomyl and imidacloprid were soaked to the tea from dried roses by boiling water. Our studies could be applied to evaluate the residue levels and dissipation of pesticides in flowers or herbs after pesticide application during the pest managements. The rapid methods allowed us to quickly inspect the residues of pesticides in different processing products for comprehensive quality control. The processing factors (i.e. the concentrated or soaked rates from fresh to dried roses and infusions) will

be applied to evaluate and revise the tolerances of pesticides in rose flowers and its derivatives.

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