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Drying and Storage Methods Affect Cyfluthrin Concentrations in Exposed Plant Samples

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Abstract Standard procedures do not exist for drying and storage of plant samples prior to chemical analyses. Since immediate analysis is not always possible, current research examined which plant drying and storage method yielded the highest cyfluthrin recovery rates compared to traditional mechanical freeze-drying methods. Fifteen mesocosms were planted with rice. Cyfluthrin (5 mg L^{-1}) was amended into the water column of individual mesocosms. 48 h later, plant material in the water column was collected from each mesocosm. Control (mechanical freeze drying) recovery was significantly greater ($p < 0.001$) than all 14 combinations of drying and storage. Significant differences also existed between all 14 different combinations. Greatest cyfluthrin recoveries in non-control plants were from the freezer-greenhouse-freezer drying and storage method. Results offer evidence for the efficient plant drying and storage methods prior to cyfluthrin analysis. Future studies should perform comparable analyses on various pesticide classes to determine possible relationships.

Keywords Pyrethroid insecticide · Analytical recovery · Pesticide · Rice

Once pesticides enter the environment, they are subject to metabolism and degradation through various physicochemical processes. While the speed of these processes depends primarily on the pesticide formulation and chemistry, researchers are still challenged with collecting and processing samples to accurately reflect pesticide exposure and contamination. Immediate sample pesticide analysis is not always possible due to logistical, equipment, or financial constraints.

Even though aqueous pesticide samples can be quickly field or laboratory extracted, other environmental samples such as plants must be ground and dried prior to extraction and analysis. Once sampled, plants can quickly lose moisture, form condensation, mold, or otherwise face microbial degradation of the pesticide (Cox 2002). There is little standardized guidance available on proper drying and/or storage methods for plant samples exposed to pesticides. Most guidance available focuses on cold storage of aqueous samples or storage of foodstuff residue analysis (Bajwa and Sandhu 2014). The European Commission Health and Consumer Protection Directorate-General (2013) issued a guidance document on pesticide residue analysis in food and feed which stated only that laboratory samples not immediately analyzed "should be stored under conditions that minimize decay." The guidance went on to state that room temperature storage (2 weeks) was sufficient for dried products, however, storage exceeding 2 weeks should be frozen (European Commission 2013). Further guidance provided in the same document stated that freezer or refrigerator storage of extracts would minimize degradation, but that analyte stability should be evaluated during method validation (European Commission 2013).

Wells and Hess (2000) noted that few detailed studies were available on storage effects of organic contaminants in biological tissues. After 1–3 days of 4°C conditions, lipid oxidation and alkylperoxide degradation begins to occur (Wells and Hess 2000). Likewise, slow freezing allows for ice formation at the cellular level, denaturing

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proteins and causing a loss of moisture (Wells and Hess 2000).

Similarly, storage can have detrimental effects on pesticide residues through various chemical and biological reactions, as well as physicochemical processes (Amvrazi 2011). While frozen pesticide samples (−10 to −20°C) are expected to be stable or decay rather slowly, humidity, photodegradation, or oxidation may affect pesticides in plant samples even at room temperature (Amvrazi 2011). Microbial activity is greatest between 10 and 45°C, conditions that may be prevalent in dry storage facilities.

Given the various challenges and factors involved in sample drying and storage methods, it is critical to develop a suitable protocol which will minimize the physical, biological, and chemical processes that may alter sample pesticide concentrations. The objective of the current study was to examine methodologies of drying (oven, greenhouse, air, chemical, or mechanical) or drying and storage (freezer or dry) that would result in the greatest recovery of cyfluthrin concentrations in plant samples when compared to the standard mechanical freeze drying method.

Materials and Methods

Fifteen, circular polyethylene mesocosms (0.41 m depth × 0.54 m radius) were filled with Lexington silt loam soil (0.25 m depth) and planted with rice (*Oryza sativa*) in a greenhouse. Once seedlings had grown to approximately 0.25 m in height, a water depth of 0.15 m was maintained in each mesocosm for a 6 weeks acclimation period. Water was monitored daily and added if necessary to compensate for evaporation. The water source was municipal well water from the city of Oxford, Mississippi.

A one-time exposure of the pyrethroid insecticide Tombstone™ (active ingredient cyfluthrin [cyano (4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate], Loveland Products, Greeley, CO) at a target concentration of 5 mg L^{-1} was amended individually into each mesocosm by pipette distribution. Water was thoroughly mixed with a glass rod, and after 15 min, water samples (500 mL) were collected from each mesocosm for cyfluthrin analysis and analyzed in duplicate. Mesocosms remained undisturbed for 48 h following cyfluthrin addition. After 48 h, only plant material exposed in the water column was collected for determination of cyfluthrin concentration. Plant material was harvested from each mesocosm, pooled together, and subsets were randomly assigned to one of 15 different drying and storage procedures for assessment. Table 1 describes the different drying and storage combinations imposed on the collected plant material. For each combination listed in Table 1, 12 replicates were analyzed [with the exception of the air dry (under a fume hood with fluorescent lighting at 210 ± 10.6 Lux), mechanical freeze dry (control), and chemical dry (alternate control) which only had six replicates each]. Control plants exposed to cyfluthrin were immediately packed on ice and sent to Mississippi State University (2 h away) for mechanical freeze drying, while chemical drying samples were accomplished on site using anhydrous sodium sulfate (Avantor Performance Materials, Paris, KY) (Blasco and Picó 2010). For initial drying methods, all plant materials were placed in aluminum pans. If samples were stored before drying, they were wrapped in aluminum foil and sealed in a plastic bag. Following grinding with a Thomas-Wiley laboratory mill (Thomas Scientific, Swedesboro, NJ), plant samples were placed in sealed polypropylene cups until final analysis.

Water (500 mL) and plant samples (2 g) for cyfluthrin analysis were assessed using methods modified by Smith and Cooper (2004) and Smith et al. (2007). Cyfluthrin was extracted from its matrix (water or plants) by way of pesticide-grade ethyl acetate. Samples were concentrated to near dryness using an Organomation OA-SYS Heating System with N-EVAP-112 Nitrogen Evaporator (Parker, Haverhill, MA). The plant extracts were cleaned using a silica gel column. Extracts were then concentrated to 1 mL using the nitrogen evaporator and water bath system.

An Agilent Model 7890A gas chromatograph (Wilmington, DE) equipped with dual Agilent 7693 autosampler and dual G4513A series autoinjectors set at 1.0 µL injection volume fast mode, dual split-splitless inlets, dual capillary columns, and Agilent ChemStation were used for cyfluthrin analyses. The Agilent 7890A GC was equipped with two micro electron capture detectors (µECDs). Carrier gas used was ultra-high purity (UHP) helium (nexAir, Batesville, MS) at 54.5 mL min^{-1} and inlet temperature at 250°C. The µECD temperature was 325°C with a constant make-up gas flow of 60 mL min^{-1} UHP nitrogen (nexAir, Batesville, MS). Detection limits were 0.05 µg L^{-1} and 12.5 µg kg^{-1} for water and plant samples, respectively.

Descriptive statistics were used to evaluate data, while statistical significance between methods was evaluated using ANOVA and Tukey's Honestly Significant Difference test with an alpha level of 0.05. JMP® 8.0.1 statistical software was used for the analyses.

Results and Discussion

Measured water cyfluthrin concentrations in the 15 mesocosms ranged from 896 (±20.5) µg L^{-1} to 1593 (±42.4) µg L^{-1}. The difference in measured aqueous concentrations and the targeted exposure (5 mg L^{-1}) was not unexpected, as cyfluthrin's octanol–water partition coefficient (K_{OW}) of

Table 1 Drying and storage combinations for plant material

Initial drying method	Time (days)	Initial storage method	Time (days)	Secondary drying method	Time (days)	Secondary storage method	Time (days)	Method combination abbreviation
OV	4	–	–	–	–	–	–	OV
OV	4	FR	30	–	–	–	–	OV, FR
OV	4	DS	30	–	–	–	–	OV, DS
GH	7	–	–	–	–	–	–	GH
GH	7	FR	30	–	–	–	–	GH, FR
GH	7	DS	30	–	–	–	–	GH, DS
–	–	FR	7	GH	7	–	–	FR, GH
–	–	FR	7	GH	7	DS	30	FR, GH, DS
–	–	FR	7	GH	7	FR	30	FR, GH, FR
–	–	FR	7	OV	4	FR	30	FR, OV, FR
–	–	FR	7	OV	4	DS	30	FR, OV, DS
–	–	FR	7	OV	4	–	–	FR, OV
AIR	7	–	–	–	–	–	–	AIR
MECH[a]	2	–	–	–	–	–	–	Control
CHEM[b]	<1	–	–	–	–	–	–	Alt. control

OV, oven (100°C); AIR, air dry (22°C for 7 days); GH, greenhouse (27–38°C); MECH, mechanical freeze dryer; FR, freezer (−10°C); CHEM, chemical dry with; DS, dry storage (24–28°C) anhydrous sodium sulfate

–None (not applicable)

[a] Method used as control

[b] Method used as alternate control

4.58×10^5–6.4×10^5 and low water solubility (2.0×10^{-3} mg L^{-1}) indicate its propensity to bind to substances such as glassware and the polyethylene mesocosms (Casjens 2008). With a Henry's Law Constant of 4.93×10^{-6} atm m^3 mol^{-1} at 20°C, cyfluthrin is less volatile than water, making loss by volatilization minimal to non-existent (Crosby 1998). This would also suggest the aqueous cyfluthrin exposure would not rapidly degrade, thus allowing time for sorption to plant material.

Although Tomlin (1997) suggested a low tendency for cyfluthrin to be translocated into plant material, results from the current study demonstrate, at a minimum, sorption of cyfluthrin to plant material. Under the current experimental design and chemical analysis methods, it was not possible to determine the difference between translocation within and sorption to plant material. Control plants (MECH) had significantly greater cyfluthrin concentrations than the other 14 drying and storage combinations (Tables 2, 3). In fact, recovery in control plants (MECH) was between 4 and 11 times greater than in the other alternative methods. Oven (only) plant samples had the lowest cyfluthrin concentrations—approximately 10 % of the control sample concentrations (Table 2). With the exception of the freezer, greenhouse, dry storage combination (Table 1), the combination of freezer storage; drying (either greenhouse or oven); followed by storage (either freezer or dry storage) resulted in higher recovered cyfluthrin concentrations in plant material. According to the FAO (2007), cyfluthrin concentrations in rice stored in the freezer for 102 days were still stable. In the current study, immediate oven drying followed by storage (either freezer or dry storage) resulted in the lowest recovered cyfluthrin plant concentrations. Wheat grain fortified with cyfluthrin and stored at 21°C lost 31 % of the pesticide after the first month, while wheat grain fortified and stored at 28°C lost 29 % of cyfluthrin after the first month. After 293 days, only 50 % of the cyfluthrin remained in the 28°C storage, while the 21°C storage had no further losses at 293 days (FAO 2007).

Significant differences were noted between the freezer, oven combination and both freezer, oven, freezer and freezer, oven, dry storage ($F_{13,142} = 20.9645$; $p < 0.0001$) (Table 3). No significant differences were noted between oven and either oven, freezer or oven, dry storage. Likewise, no significant differences were noted between greenhouse and either greenhouse, freezer or greenhouse, dry storage (Table 3). Garrido-Frenich et al. (2003) sampled pesticide amended fresh and dry leaves and stems from French bean, melon, and watermelon in greenhouses, conserved samples at 4°C until extraction, then stored samples at −20°C in the dark until analysis. Results indicated no degradation of pesticides under these storage

Table 2 Measured mean (±SE) cyfluthrin concentrations in plant material

Initial drying method	Initial storage method	Secondary drying method	Secondary storage method	Method combination abbreviation	Cyfluthrin (mg kg^{-1})	Equivalent factor[a]
OV	–	–	–	OV	17.2 (0.89)	0.091
OV	FR	–	–	OV, FR	23.6 (1.31)	0.126
OV	DS	–	–	OV, DS	23.9 (2.63)	0.127
GH	–	–	–	GH	31.3 (2.00)	0.166
GH	FR	–	–	GH, FR	33.1 (2.18)	0.176
GH	DS	–	–	GH, DS	29.8 (1.65)	0.159
–	FR	GH	–	FR, GH	32.0 (1.30)	0.170
–	FR	GH	DS	FR, GH, DS	29.4 (1.15)	0.156
–	FR	GH	FR	FR, GH, FR	42.0 (1.70)	0.223
–	FR	OV	FR	FR, OV, FR	35.9 (1.37)	0.191
–	FR	OV	DS	FR, OV, DS	36.9 (0.73)	0.196
–	FR	OV		FR, OV	23.8 (1.11)	0.127
AIR				AIR	24.2 (0.98)	0.129
MECH				Control	188 (45.8)	–
CHEM				Alt. control	33.8 (2.59)	0.180

N = 12 for all samples except AIR, MECH, and CHEM where N = 6

OV, oven; AIR, air dry; GH, greenhouse; MECH, mechanical freeze dryer; FR, freezer; CHEM, chemical dry; DS, dry storage

–None (not applicable)

[a] Equivalent factor: $[\text{cyfluthrin}]_{\text{method}}/[\text{cyfluthrin}]_{\text{mechanical control}}$

Table 3 Significant differences (alpha = 0.05) between drying and storage combinations using Student's *t* test and Tukey HSD

	OV	OV, FR	OV, DS	GH	GH, FR	GH, DS	FR, GH	FR, GH, DS	FR, GH, FR	FR, OV, FR	FR, OV, DS	FR, OV	AIR	MECH	CHEM
OV				*	*	*	*	*	*	*	*			*	*
OV, FR				*	*		*		*	*	*			*	*
OV, DS				*	*		*		*	*	*			*	*
GH	*	*	*						*			*		*	
GH, FR	*	*	*						*			*	*	*	
GH, DS	*								*		*			*	
FR, GH	*	*	*						*			*		*	
FR, GH, DS	*								*		*			*	
FR, GH, FR	*	*	*	*	*	*	*	*				*	*	*	
FR, OV, FR	*	*	*									*	*	*	
FR, OV, DS	*	*	*			*		*				*	*	*	
FR, OV				*	*		*		*	*	*			*	*
AIR					*				*	*	*			*	
MECH	*	*	*	*	*	*	*	*	*	*	*	*	*		*
CHEM	*	*	*									*		*	

Significance indicated by asterisk. Refer to Table 2 for mean cyfluthrin values to determine which method is more efficient

OV, oven; GH, greenhouse; FR, freezer; DS, dry storage; AIR, air dry; MECH, mechanical freeze dry; CHEM, chemical dry

conditions for at least 6 months (Garrido-Frenich et al. 2003). Greenhouse (only) was significantly better than oven; oven, freezer; oven, dry storage; and freezer, oven ($F_{13,142} = 20.9645$; $p < 0.0001$) (Table 3). Greenhouse (only) was significantly less than freezer, greenhouse, freezer ($F_{13,142} = 20.9645$; $p < 0.0001$).

There was not a significant difference between AIR and either oven; oven, freezer; or oven, dry storage. Chemical drying was significantly better than oven; oven, freezer; oven, dry storage; and freezer, oven ($F_{13,142} = 20.9645$; $p < 0.0001$) (Table 3). Greenhouse (only) was significantly better than oven; oven, freezer; oven, dry storage; and freezer, oven ($F_{13,142} = 20.9645$; $p < 0.0001$) Greenhouse (only) was significantly less than freezer, greenhouse, freezer ($F_{13,142} = 20.9645$; $p < 0.0001$) (Table 3).

Mechanical freeze drying of plant samples is not always an option for researchers due to budget or logistical (e.g. remote field locations or long field travel distances) constraints. For cases when mechanical freeze drying is unavailable, freezer, greenhouse, freezer would be the best option among the combinations examined in the current study. At the USDA Agricultural Research Service National Sedimentation Laboratory, researchers were historically utilizing the method of freezer, greenhouse, dry storage due to sample analysis backlog and absence of equipment for mechanical freeze drying. In the evaluation of methods, freezer, greenhouse, dry storage is still significantly better than oven, but significantly less than freezer, greenhouse, freezer and freezer, oven, dry storage ($F_{13,142} = 20.9645$; $p < 0.0001$) (Table 3). If mechanical freeze drying equipment is not available, researchers must choose the best available options for drying and potential storage of samples until pesticide analyses. Heat degradation, enzymatic transformations, metabolism, photodegradation, and volatilization (Amvrazi 2011) should all be taken into consideration when deciding upon drying and storage methods for pesticide-amended plant material. Based on the current research results, alternative methods to sample drying and storage will probably reduce the likelihood of detecting cyfluthrin concentrations compared to the control technique, given the large differences in recoveries. Since little information is available in the literature regarding the most effective drying and storage methods for pesticide-exposed plant samples, more studies should be conducted to determine best methods for different pesticide classes.

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