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Fenitrothion and esfenvalerate stability during corn and wheat sample processing

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Abstract

The effects of three processing methods for corn and wheat samples on the stability of stored-grain protective insecticides were evaluated. Fenitrothion and esfenvalerate were applied so as to produce theoretical concentrations of 10 and 0.5 mg kg^{-1}, respectively. Two hours after treatment, the grains were processed and deposition was analyzed by gas chromatography. Grain species did not influence insecticide stability. This stability was only dependent upon processing method and insecticide. Grains processed together with dry ice provided the greatest percentage of recovery for both insecticides. Regardless of processing method used, more esfenvalerate than fenitrothion was recovered, thus demonstrating the greater stability of the pyrethroid during this operation. The factors that influenced insecticide stability are discussed in the present work.

Key words: Stored grain, insecticide degradation, dry ice, gas chromatography, uncertainty

Introduction

The presence of insecticide residues in cereals represents a risk for the consumer, because these substances are used in large scale to protect stored grains from the attack of pests. Therefore, to prevent human populations from consuming foods with dangerous levels of these substances, every registered product is accompanied by a maximum residue limit (MRL) allowed by law for each agricultural product. The risk becomes more apparent when laboratories specialized in residue analysis show inconsistent results, as a consequence of a high degree of uncertainty in the reliability of the analytical procedures.

In pesticide residue studies on plant materials, the phase that precedes the analytical step can be divided into sample processing and subsampling (Silva et al., 2003). Sample processing is one of the steps that can significantly influence the uncertainty and precision of results, because during this stage the pesticides may become volatilized and subject to hydrolysis and enzymatic reactions (Ambrus, 2004). This loss may lead to underestimation

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errors of the amount of residue present in food, and may put consumer health at risk.

The organophosphorus insecticide fenitrothion and the pyrethroid esfenvalerate are among the insecticides registered in Brazil for stored grain treatment (Agência Nacional de Vigilância Sanitária, 2005). Both insecticides have different physicochemical properties; these properties may influence their stability during sample processing in machines that generate heat. The objective of this work was to evaluate the effects of three processing methods for corn and wheat grains on the stability of fenitrothion and esfenvalerate.

Material and methods

The experiment was conducted at the Pesticide Residue and Chromatographic Analysis Laboratory of Departamento de Entomologia, Fitopatologia e Zoologia Agrícola of Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ/USP), in Piracicaba, SP, Brazil.

Grain treatment

Corn and wheat cultivars Sol-da-Manhã and BRS 208 were used, respectively, both developed by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA - Brazilian Agricultural Research Corporation). Fenitrothion and esfenvalerate were applied so as to produce theoretical concentrations of 10 and 0.5 mg kg^{-1}, respectively. The trade product Sumigranplus® (500 g of the a.i. fenitrothion + 25 g of the a.i. esfenvalerate/liter) was used.

For treatment purposes, the corn and wheat were packaged into plastic bags. The application was performed with a sprayer attached to an air compressor with a constant pressure of 150 kPa, using 22.5 mL of insecticide emulsion (1:250 dilution of the commercial product in water) for 4.5 kg of grain (5 L t^{-1}). During the spraying operation, the bags were agitated manually, allowing the mix to be distributed as homogeneously as possible. The same procedure was adopted for the control treatment, but in this case the spray consisted of water only. The temperature and relative humidity during spray were 26.2 °C and 87 %, respectively.

Sample processing

Two hours after spraying, 0.5 kg of corn and wheat grains were collected and processed by one of the three processing methods: grains ground without dry ice, grains ground with dry ice, and grains without processing. Three replicates were made, generating 18 experimental plots, and two insecticides were analyzed, totaling 36 subplots. In the first two methods, the grains were ground in a model TRF70 forage chopper; in the second method, the dry ice was mixed with the grains at a 1:1 ratio prior to grinding. In the third method, the whole grains were taken directly to the laboratory for the respective analysis. The temperature and relative humidity during processing were 29.1 °C and 86 %, respectively.

Analytical procedure

The analytical method was adapted from Ohlin (1998). Ten grams of homogenized samples were placed in 100 mL Schott bottles for residue extraction. Fifty mL ethyl acetate and 10 g sodium sulfate were added and later homogenized in a stirring table for 1 hour at 360 cycles min^{-1}. After this operation, the extracts were centrifuged for 5 min at 2,600 rpm for better separation of the liquid phase from suspension materials. Ten milliliters aliquots of the supernatant were transferred to 12 mL test tubes, corresponding to 2 g of the original sample, and were then added of 50 mL dodecane. The extracts were evaporated in a Turbo-Vap evaporator, in water bath at 30 °C aided by moving air previously dried through a blue silica gel desiccant filter. The insecticide residues were then resuspended in 5 mL of a cyclohexane / ethyl acetate mixture (1:1, v/v), homogenized in vortex mixer/ultrasound and filtered through a Millipore, FG, 0.2 mm pore

membrane filter mounted on a plastic hypodermic syringe (5 mL). The extracts were cleaned by gel permeation chromatography (GPC) and eluted with a cyclohexane / ethyl acetate mixture (1:1, v/v). After this operation, the extracts were evaporated in a Turbo-Vap evaporator previously added of 50 mL dodecane and were later resuspended in 20.0 and 1.95 mL of the cyclohexane / ethyl acetate mixture (1:1, v/v) for the fenitrothion and esfenvalerate residues, respectively.

The samples were analyzed by gas chromatography, using a Thermo Quest gas chromatograph, model Trace, equipped with an electron capture detector (ECD, Ni^{63}) and capillary chromatographic column Restek Corp. RTX-5MS, 30 m length, 0.25 mm internal diameter, and 0.25 mm film thickness, with injections made in the splitless mode. The chromatograph was operated under the following conditions: column temperature = 100 °C (initial); then at 280 °C (25 °C min^{-1} ramp), remaining at this temperature for a period of ten minutes; injector temperature = 230 °C; detector temperature = 320 °C, purge time = 0.75 minute; gas flow (mL min^{-1}): H_2 (carrier) = 1.2; N_2 (make up) = 45 and purge flow = 65; run time = 18 minutes and 15 seconds. Under these conditions, retention time was 7 minutes and 5 seconds for fenitrothion and 12 minutes and 40 seconds for esfenvalerate, approximately. The residues were calculated using the ChromQuest version 4.1 software, based on a previously plotted calibration curve obtained from injections of 5, 10, 20, 40, 100, and 200 pg of fenitrothion and esfenvalerate analytical standard into the chromatographic system.

Validation of the analytical method

The analytical method used for corn and wheat grains was validated by means of matrix fortification at the levels of 0.05, 0.5, and 10.0 mg kg^{-1} for fenitrothion and 0.05, 0.1, and 1.0 mg kg^{-1} for esfenvalerate, with three replicates for each level (nine fortified samples for each matrix). Recoveries between 70-120 % were considered acceptable.

Statistical analysis

The data were submitted to analysis of variance, using a mathematical model for a completely randomized design in a split-plot arrangement, and the F test was used to evaluate the significance of factors (grain species, processing method, insecticide and interactions) in the model (Steel and Torrie, 1960; Pimentel-Gomes, 1987). Because the processing method factor was a qualitative variable with three levels, whenever the F test detected a significant difference between its means or between the means of the interaction, a detailed analysis was obtained by the Tukey test, considering a minimum significance level of 5 % ($P < 0.05$). Since the grain species and insecticide factors have only two levels, the F test in the analysis of variance is already conclusive.

Results

The insecticide recovery percentages in the fortified corn and wheat grains were acceptable (70-120 %), thus validating the analytical method. None of the two insecticides was recovered from the control, indicating that the grains were free from contamination by those compounds.

The analysis of variance only detected significant effects ($P < 0.05$) for processing method and insecticide (Table 1). Neither grain species nor any of the interactions influenced insecticide stability, which was dependent only upon insecticide and processing method when used individually. The processing method where the grains were ground mixed with dry ice provided the highest recovery percentage in both insecticides, and was significantly different from the other two methods by (Table 2). Regardless of processing method used, more esfenvalerate than fenitrothion was recovered, thus demonstrating the greater stability of the pyrethroid when compared with the organophosphorus insecticide.

Table 1. Analysis of variance for insecticide recovery percentage in corn and wheat grains.

Cause of variation	DF[1]	SS[2]	MS[3]	F	Pr > F
Grain species	1	21.16	21.16	0.26	0.6223
Processing method	2	3397.45	1698.73	20.52	0.0001
Grain species × Method	2	469.13	234.56	2.83	0.0982
Residue (A)	12	993.52	82.79		
Plots	(17)	(4881.26)			
Insecticide	1	4480.07	4480.07	41.50	<0.0001
Grain species × Insecticide	1	176.00	176.00	1.63	0.2258
Method × Insecticide	2	21.67	10.84	0.10	0.9052
Species × Method × Insecticide	2	412.68	206.34	1.91	0.1903
Residue (B)	12	1295.39	107.95		
Total	35	11267.08			

[1] DF = Degrees of freedom;

[2] SS = Sum of squares;

[3] MS = Mean square.

Table 2. Means and standard errors for insecticide recovery percentage in corn and wheat grains as a function of processing method and insecticide.

	Processing Method		
Insecticide	No Processing	Without Dry Ice	With Dry Ice
Esfenvalerate	105.7 ± 4.24 bA	97.7 ± 4.24 bA	122.3 ± 4.24 aA
Fenitrothion	82.2 ± 4.24 bB	77.6 ± 4.24 bB	99.0 ± 4.24 aB

Means followed by different lower case letters in the rows differ significantly by Tukey test ($P < 0.05$); means followed by different upper case letters in the columns differ significantly by F test ($P < 0.05$).

Discussion

It can be seen that esfenvalerate recovery was higher than 100 % in non-processed grains and in those processed with dry ice. Later, a flow increase was verified in the spraying system, even when working at constant pressure, therefore producing higher deposits than those intended. The problem verified during spray does not compromise the results of this experiment, because only one spray was performed for each grain species, and then the samples were collected and processed by one of the three methods studied. In view of this, all treatments (processing methods) received the same amount of insecticide. The later recovery of insecticides was dependent exclusively on processing method and on the physicochemical characteristics of the molecule.

The mean temperatures in the samples before and after grinding without dry ice were 25 and 35 °C, respectively. The temperature increase during sample processing was one of the factors responsible for degradation of both insecticides. According to Rowlands (1967), the temperature in the grain has a significant effect on the velocity of reactions catalyzed by enzymes, and a 10 °C increase could double the rate of these reactions. Losses between 40 and 70 % in the theoretical rates of captan, captafol, folpet, chlorotalonil, and dichlofluanid were found in fruits and vegetables

processed at room temperature (El-Bidaoui et al., 2000; Hill et al., 2000). The results described here coincide with those obtained by Fussell et al. (2002), which demonstrated the stability of 94 pesticides during processing of apple fruit frozen in the presence of dry ice (cryogenesis).

On the other hand, studies on insecticide effectiveness and residues in stored grains can be found in the international literature showing small deposits right after spray, but with high effectiveness in the control of pests. This would be due in part to a loss of insecticides during sample processing at room temperature with a consequent underestimation of the deposit.

Non-processed grains showed similar results to those obtained by grinding without dry ice, for both insecticides. The explanation for this result is that the insecticides penetrated whole grains, and were partially extracted during the extraction procedure. The insecticides we studied are contact insecticides; even so, they can penetrate and even move within the plant tissues (Finlayson and MacCarthy, 1965). The rate at which contact insecticides penetrate stored grains affects their metabolic fate and the persistence of their residues, with degradation being directly proportional to penetration velocity (Rowlands, 1971). In addition to the physicochemical characteristics of the insecticide, formulation has great influence on penetration; lipophilic insecticides can penetrate more easily when formulated as emulsifiable concentrates (Ebeling, 1963), as were the insecticides in our study.

The following could be mentioned among the most important physicochemical characteristics for the stability of these insecticides: vapor pressure, with values of $2.0\ 10^{-7}$ and $1.8\ 10^{-2}$ Pa; n-octanol-water partition coefficient (K_{ow}), with logarithmic values of 6.22 and 3.43; solubility in water, with values of 0.002 and 21 mg L^{-1}, and molecular weight, with values of 419.9 and 277.2 for esfenvalerate and fenitrothion, respectively (Tomlin, 1995). A greater vapor pressure means that the substance is more volatile; this explains the higher fenitrothion losses during spray and sample processing. Processing machines that produce temperature increases during this operation cause greater losses, especially of more volatile pesticides. At smaller log K_{ow} values, the substance is more hydrophilic, and the more water-soluble the insecticide, the more easily it will penetrate the grain, thus increasing its degradation rate. Therefore, the smaller fenitrothion recovery in the non-processed grains was due to the solvent's incapability to extract the insecticide from within itself, in addition to its higher degradation. Rowlands (1966) observed that 50 % of a fenitrothion dose penetrated the wheat grain endosperm one hour after treatment; after two hours, half of the insecticide inside the grain had been degraded by acid phosphatases. Molecular size should also be taken into consideration in the estimation of insecticide solubility in water; as a rule, large molecules are less soluble than small molecules (Seiber, 1999); as a result, fenitrothion is more soluble than esfenvalerate. On the other hand, high temperatures increase the rate of degradation because substances become more soluble (Stenersen, 2004).

High uncertainty in analytical procedures, especially in the field of pesticide residues, where the detection of very small amounts of pesticides is sought, may lead to results that are at times unreliable and doubtful. The results here reported demonstrate that the use of dry ice in sample processing procedures for pesticide residue analyses is highly recommended if greater confidence and precision of analytical results is desired.

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