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Short communication

Adaptation of the internal standard method to a pesticide residues study in/on plants

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Abstract

The aim of the trials was to adapt the principles of the internal standard technique for the study of disappearance of pesticide residues. Tomato and pepper plants were treated with homogenous mixtures of two or three agricultural chemicals. Hence, it was stated that 5 and 24 h after treatment, heptenophos residues remained at 25 and 5% of those amounts present on tomatoes at hour 0 while those of pirimiphos-methyl were 100 and 88%, respectively. Heptenophos residues disappeared more quickly on green pepper fruits than on red pepper fruits. Comparative study seems to be a simple and reliable method for the degradation behavior of pesticide residues.

Keywords: Tomatoes; Environmental analysis; Pesticides

1. Introduction

Chromatographic quantitative analysis is based upon two requirements: reproducible measurements and linear response of the detector for the determined compounds. When these criteria have been met, and the peaks have been quantified, the chemist now has numbers representing peak heights or areas. In order to transform these numbers (raw data) into residue values, there are three different mathematical manipulations: area normalization, external standard and internal standard technique. The latter requires the addition to the sample of an internal standard for comparative determination. The unknown concentration of the determined compound is then read from the internal standard calibration curve plotted with the peak height (or area) ratio of the compound of interest to the internal standard vs. concentration of the former. Since the amount of internal standard added as well as the final volume of an extract are constants, certain errors may be compensated.

On the other hand, however, uneven coverage of surfaces of the same and of different plants with a chemical during treatment causes large differences in the results of the analyses of samples taken from the same plot in several replications. It is therefore understandable that even under a correct survey procedure, such as that by Cabras et al. [1,2], pesticide residues may exceed a few days after spraying the level of their initial values. The effect of plant growth on the course of these changes also seems to be underestimated because it is only in recent years that some researchers reported, along with the results of their chemical analyses, the mass of tested plants or of their edible parts [3,4]. These factors constitute the main obstacles in the evaluation of the cause of the variations in pesticide content described in literature, which are not always consistent with the results achieved, due to degradation, dissipation, disappearance, dilution or decay.

The aim of the study was to adapt the principles of the internal standard technique for the evaluation of

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the disappearance of pesticide residues independent both of uneven coverage of tomatoes during treatments and of dilution effect caused by fruit growth.

2. Experimental

2.1. Experiment I

The field trial was carried out in a commercial greenhouse of the Regional Quarantine and Plant Protection Station at Rzeszów, where four adjoining rows, containing 24 tomato plants, equivalent to 0.001 ha, were separated. The plants were sprayed early in the morning with a homogenous aqueous mixture of Nurelle 550 EC (50% of chlorpyrifos+ 5% of cypermethrin), Actellic 50 EC (50% of pirimiphos-methyl) and Hostaquick 500 EC (50% of heptenophos) using for its preparation 0.001 of the amount for a hectare of this crop at concentrations recommended by the Plant Protection Institute at Poznań. Sampling started about 10 min after spraying. Each time four tomatoes were taken from randomly selected plants and then separately analyzed immediately after sampling.

2.2. Experiment II

The field trial was carried out in an experimental greenhouse of the Horticultural Institute at Skierniewice. The pepper plants were treated in the afternoon with a homogenous mixture of Actellic 50 EC (50% of pirimiphos-methyl) and Hostaquick 500 EC (50% of heptenophos) in a mineral oil [5] by a fogging method at rates recommended by the Plant Protection Institute at Poznań. Sampling started in the morning of the next day. Each time four average pepper samples were taken and then 100 g subsamples were weighed into a 250 ml screw-capped flask and frozen at −20°C. After the trial was completed, the frozen subsamples were transported in dry ice by car to the Institute of Plant Protection Laboratory at Rzeszów for the residue analysis.

2.3. Analytical procedure

Chopped fresh tomato fruits or pepper subsamples were homogenized with acetone and a one-fifth volume of water–acetone filtrate was placed in a separatory funnel and shaken three times (20 and 2×10 ml) with dichloromethane [6,7]. The extract obtained, equivalent of 20 g of the sample, was evaporated to dryness with rotary evaporator Rotavapor-R and residues were dissolved in *n*-hexane and diluted in a calibrated flask to 25 ml. No clean-up of the extract was required.

Analyses were carried out with the use of a Pye Unicam 104 gas chromatograph equipped with a thermionic detector on a glass column (90 cm×0.4 cm) with 3% of OV-101 on Gas-Chrom Q, 80–100 mesh. In the case of Experiment I, peak heights (peak areas may also be used) were measured because only relative changes of heptenophos and pirimiphos-methyl contents in comparison to stable chlorpyrifos were to be estimated (Fig. 1). In the case of Experiment II, quantitative determinations of heptenophos and pirimiphos-methyl residues were performed by external standard technique through measurements of peak heights of a standard solution and of tomato extracts at isothermal conditions in the linear range of detector responses (below 0.2–0.5

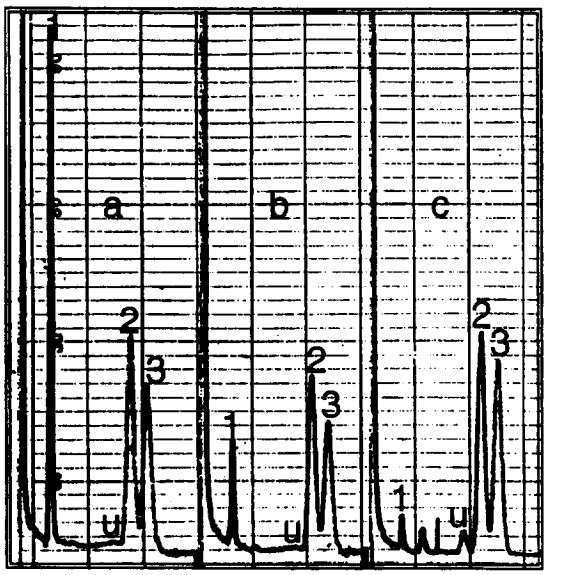

Fig. 1. Representative thermionic detection chromatograms obtained for tomato samples: (a) 0 h after treatment, (b) 5 h after treatment and (c) 24 h after treatment; (1) heptenophos, (2) pirimiphos-methyl, (3) chlorpyrifos and (u) unknown compound – probably a breakdown product of pirimiphos-methyl; Experiment I.

ng). For all compounds the average recoveries from fortified samples were greater than 93% with standard deviations less than 5%. Tomatoes, as well as solvents, did not contain impurities which would interfere with the determined compounds.

2.4. Mathematical calculations

The peak height data obtained in the degradation study of heptenophos, pirimiphos-methyl and chlorpyrifos (Experiment I) are summarized in Table 1. These data were subjected to mathematical calculations using the equation

$$[PHR][(PHR)_0]^{-1}100\%$$

where PHR is the peak height ratio of heptenophos (or pirimiphos-methyl) to chlorpyrifos (assumed as a stable internal standard) at t hours after application, $(PHR)_0$ is the average peak height ratio of heptenophos (or pirimiphos-methyl) to chlorpyrifos at time $t=0$. Thus, the percentages of initial amounts of heptenophos and pirimiphos-methyl present at the moment of sampling on a given tomato fruit were obtained. In Table 1, only their average values are given.

The residue data obtained in the degradation study of heptenophos and pirimiphos-methyl (Experiment II) are summarized in Table 2. These data were subjected to mathematical calculations using the equation

$$[RR][(RR)_0]^{-1}100\%$$

where RR is the residue ratio of heptenophos to pirimiphos-methyl (assumed as a stable internal standard) at t days after application, $(RR)_0$ is the greatest (not an average) residue ratio of heptenophos to pirimiphos-methyl at time $t=0$. Thus, the percentage of initial amount of heptenophos present at the moment of sampling on given pepper fruits was obtained. In Table 2, their particular values are added.

3. Results

3.1. Experiment I

The active ingredients of used chemicals belong to the group of organophosphorus insecticides. Both the literature data, and many years of experience gained from monitoring surveys, indicated that pirimiphos-methyl and chlorpyrifos were the compounds which remained longer on plants after treatment [5,8]. In the present study, chlorpyrifos appeared more persistent and was assumed as the basis (internal standard) for the evaluation of the behavior of the two remaining ones.

Immediately after treatment, peak height ratios of heptenophos to chlorpyrifos ranged from 3.0 to 3.3 (on average 3.15) and were equal to the ratio between their concentrations in the spray solution. They also indicated good mixing of the chemicals and enabled an exact evaluation of the rate of degradation process. Hence, 5 and 24 h after treatment, heptenophos amounts as well as concentrations (no dilution effect) constituted, respectively, 25 and 5% of those present on tomatoes at hour 0. Peak

Table 1
Peak heights (mm) and average percentages of initial amounts of heptenophos and pirimiphos-methyl in comparison to chlorpyrifos in tomatoes, Experiment I

Hours after treatment	Heptenophos 1/2/3/4	Pirimiphos-methyl 1/2/3/4	Chlorpyrifos 1/2/3/4
0	93.0/73.5/75.0/71.0 100	39.0/30.0/30.5/28.5 100	31.0/23.5/23.5/21.5 100
5	17.5/16.5/13.0/17.5 25	29.0/27.5/25.5/23.0 100	22.5/20.5/20.0/18.0 100
24	6.0/5.0/5.0/3.0 5	40.0/29.0/73.0/19.0 88	35.0/25.0/59.0/16.0 100

Peak heights were measured after adjusting concentrations of the compounds in the extracts to similar levels.

Table 2
Residues (mg/kg) and percentages of initial amounts of heptenophos in comparison to pirimiphos-methyl in peppers, Experiment II

Days after treatment	Heptenophos 1/2/3/4	Pirimiphos-methyl 1/2/3/4
1	0.14/0.12/0.07/0.06 100/63/65/32	0.25/0.35/0.18/0.33
3	0.12/0.05/0.13/0.04 57/33/52/21	0.39/0.31/0.46/0.35
5	0.05/0.04/0.02/0.02 46/22/16/15	0.22/0.36/0.22/0.31
7	0.08/0.08/0.02/0.01 46/45/14/2	0.32/0.32/0.23/0.31
9	0.03/0.03/0.01/n.d. 17/12/5/<1	0.32/0.38/0.29/0.36
11	0.02/trace/n.d./n.d. 23/1/<1	0.12/0.25/0.23/0.23
13	0.02/n.d./n.d./n.d. 12/<1	0.23/0.29/0.31/0.32

Results were ordered according to colour of pepper extracts (from red, through red-green and green-red to green).

height ratios of pirimiphos-methyl to chlorpyrifos also were in a similarly narrow range from 1.26 to 1.33 (on average 1.29). Their values, however, indicated that pirimiphos-methyl amounts as well as its concentrations stayed at the same levels for 5 h after treatment and then dropped by just 12% during the first 24 h. Therefore, the obtained results are clear proof that the cause of reduction of heptenophos deposits was its rapid degradation and/or volatilization from tomatoes, while those of pirimiphos-methyl underwent only a small degree of decomposition in the same period.

3.2. Experiment II

Some relationship between residue levels and the colours of the extracts was observed after the first injections. Hence, the colours were noted in a simplified manner as red, red-green, green-red and green. It was also found that *RR* values of heptenophos to chlorpyrifos, generally higher in the extracts of red colour, immediately after treatment were in a broader range than *PHR* values of experiment I, despite the fact that in both experiments the two chemicals were applied in the form of homogeneous mixtures. This led to the supposition that, especially on green peppers, the process of heptenophos disappearance started just after the treatment. Therefore, in the case of experiment II, not the average but the highest residue ratio of heptenophos to pirimiphos-methyl, found in an extract of red colour, seemed to be close or equal to the ratio between their concentrations in spray solution and that one was chosen as a basis for the evaluation of heptenophos disappearance. Heptenophos content after treatment constituted, depending on the colour of the extract, from 30 to 100% of its initial amount and then in red pepper dropped by half within the first 7 days (half-breakdown time) while in green pepper it dropped to trace level (not more than 2% of the initial amount). This may indicate that degradation rate depends on the ripeness of pepper.

4. Discussion

A comparative study seems to be the only simple and reliable method for the estimation of the behavior of a pesticide after application. The study makes it possible to determine real disappearance of pesticide residues independently from uneven coverage of plant surface and of plant growth. The results achieved are as reliable as those obtained by the analytical method using the internal standard tech-

nique for quantitative analysis. For proper selection of internal standard the following criteria must be met: (1) complete resolvement from sample component peaks, (2) persistent within sufficient period of time, (3) linear with a compound tested in the concentration range of interest, (4) similar concentration range to that of the compound tested, and (5) simultaneous determination with the tested compound at isothermal conditions.

The above conditions are met by (1) chlorpyrifos which may be a reference compound for the majority of organophosphorus compounds, for the carbamates as well fungicides such as chlorothalonil, dichlofluanid, vinclozolin, procymidone, bupirimate, pyrifenox etc. and (2) bromopropylate or iprodione which seem to serve as good internal standard for the synthetic pyrethroids, tetradifon, methoxychlor pyrazophos, phosalone, etc. [9]. The proposed method may be applied with success during the registration procedure of agrochemicals.

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