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Methodology for the estimation of chlorothalonil and its metabolite in mustard crop by gas liquid chromatography

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Abstract. A simplified method for the determination of chlorothalonil (I) and its metabolite 4-hydroxy-2,5,6-trichloro-isophthalonitrile (II) in mustard crop is described. It involves extraction, derivatisation, clean-up on a silica-gel column and gas-liquid chromatography. The retention times and detection limits are 4.49, 5.39 min and 0.01, 0.005 μg/g for I and II, respectively.

Chlorothalonil (2, 4, 5, 6-tetrachloro-isophthalonitrile, I) is a broad spectrum fungicide. It was found effective in controlling Alternaria blight disease in mustard (*Brassica campestris* L.) but its residues [1–4] have not been determined on this crop. Since pesticides are known to accumulate [5] in the seed of this oil-bearing crop and the acceptable daily intake (0.0005 mg/kg) of I is relatively low, a suitable method is required for the estimation of chlorothalonil and for studying its persistence on mustard.

4-Hydroxy-2,5,6-trichloro-isophthalonitrile (II) is known to be formed in crops following the application of I [3]. II does not respond to gas liquid chromatography (GLC) and has to be derivatised [6] before its analysis. Most of the published methods [4, 7] therefore estimated only the parent compound (I). The method of Tillman [7] required a not readily available 1-methyl-3-p-tolylthiazene reagent and others gave less than the desired recovery from mustard seeds. Hence the published methods [8, 9] were not found suitable for the estimation of I and II in mustard crop unless they are modified. Therefore, a modified method for this purpose has been worked out.

The average percentage recovery of I and II from mustard leaves, pod covers and seeds are given in Table 1. It ranged from 80–97%. Precision of the method is reflected from the standard deviation data presented in the table.

Experimental

Chlorothalonil Dust Formulation 72 WP was supplied by M/s. Sandoz, India. The pure sample of I needed for the study was obtained by extracting 72% WP formulation with methanol followed by recrystallisation (mp 250–251°C).

II was synthesised by refluxing I (1 g) with ethanolic potassium hydroxide (5%, 20 ml) for 4 h. After removing ethanol by rotary evaporation, the reaction mixture was acidified with dilute hydrochloric acid and extracted with ethyl acetate. The organic layer after washing with water was evaporated under reduced pressure and the solid obtained was recrystallised from methylene chloride to get a pure sample of II (m.p. 101°C); MS (M^+ 248, M + 2 250).

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Table 1. Percent recovery of chlorothalonil (I) and 4-hydroxy-2,5,6-trichloro-isophthalonitrile (II) from mustard crop[a]

Substrate	Fortification level μg/ml	Percent recovery chlorothalonil	Percent recovery 4-hydroxy-2,5,6-trichloro-isophthalonitrile
Mustard leaves	0.5	92.6 (3.8)[a, b]	85.6 (4.3)[b]
	1	96.8 (2.5)	90.4 (2.2)
Pods	0.5	90.4 (2.5)	83.5 (4.7)
	1	88.6 (5.1)	86.2 (3.4)
Pod covers	0.5	94.6 (4.3)	80.1 (2.8)
	1	96.0 (2.9)	82.6 (4.1)
Seeds	0.5	89.6 (4.1)	85.4 (3.8)
	1	91.2 (3.2)	88.7 (4.3)

[a] Initial residues on leaves were 12.36 μg/g and in harvest time seed they were non-detectable (< 0.01 for I and 0.005 μg/g for II) following application of the fungicide (630 g a.i./ha)
[b] Standard deviation (±) of 3 replicates is given in parentheses

Procedure. Field samples of mustard leaves, pods, pod covers and seeds were separately fortified with I and II at 0.5 and 1 μg/g levels in triplicate. The leaf samples (50 g) and pods (50 g) were homogenised with 50 ml acetone containing 5 ml of orthophosphoric acid (85%) in a Waring blender for 3 min thrice. The acetone extract was rotary evaporated to dryness and a saturated solution of sodium chloride (150 ml) was added. The extract was partitioned thrice into chloroform (50 ml) and then processed for clean-up and derivatisation. The chloroform extract was completely evaporated under reduced pressure and diethyl ether (5 ml) was added, followed by an ethereal solution of diazomethane (5 ml). The yellow solution was left for 3 h and then evaporated. The concentrate was passed through a prewashed (30 ml, hexane-acetone, 9 : 1 V/V) column of silica gel (6 g) placed between layers of sodium sulphate (1 g). I eluted with hexane – acetone (100 ml, 50:1, V/V) and II as the methyl ether with hexane-acetone (100 ml, 20:1 V/V). The eluates were concentrated and analysed by GLC.

Gas liquid chromatography. The estimation was carried on a Varian 3400 GLC fitted with an electron capture detector and a glass column (2 m long, 2 mm i.d.) packed with 3% OV-25 on Chromosorb WHP 80–100 mesh. The column was maintained at 190°C, injection port and detector were set at 210°C and 250°C, respectively. The carrier gas flow rate was 30 ml/min. The retention times of I and II were 4.49 and 5.39 min., respectively. The limit of detection was 0.01 μg/g for I and 0.005 μg/g for II.

The identity of the compounds was confirmed by carrying out analyses on HP-5890 GLC fitted with a megabore column (HP-17) and by mass spectroscopy.

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Comparison of different extraction methods for the determination of polycyclic aromatic hydrocarbons in soil

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Abstract. Two different extraction methods for the determination of polycyclic aromatic hydrocarbons in soil are compared: the extraction in combination with ultrasonic treatment, and the Soxhlet extraction method according to DIN-draft 38414 Part 21. Different types of real soil were extracted and analysed by HPLC with diode-array detector and fluorescence detection. The results show that the efficiency of the ultrasonic method is comparable to the Soxhlet method.

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1 Introduction

The determination of PAH in soil is usually carried out by Soxhlet extraction [1–3]. The disadvantages of this method are a large solvent consumption as well as a long sample treatment. We developed an ultrasonic extraction method in order to avoid these disadvantages. In this new method ultrasonic waves in a liquid bath enhance the extraction effect owing to the phenomenon of the so-called cavitation [4].

2 Experimental

For the extraction of polycyclic aromatic hydrocarbons three different matrices were examined: loam, humus and sand.

Ultrasonic method. The dry soil was shortly shaken with 10 ml tetrahydrofuran to provide a better contact of solvent and soil, so that the equilibrium of the PAH is established between the solid and the liquid phase, extracted for 1 h in the ultrasonic bath and then shaken again. In order to separate disturbing matrix components, columns filled with benzenesulfonic acid modified silica gel (BSA) were used to clean the extract [5].

Soxhlet method. Dry soil was extracted in the Soxhlet apparatus with cyclohexane. The extraction cycles took approximately

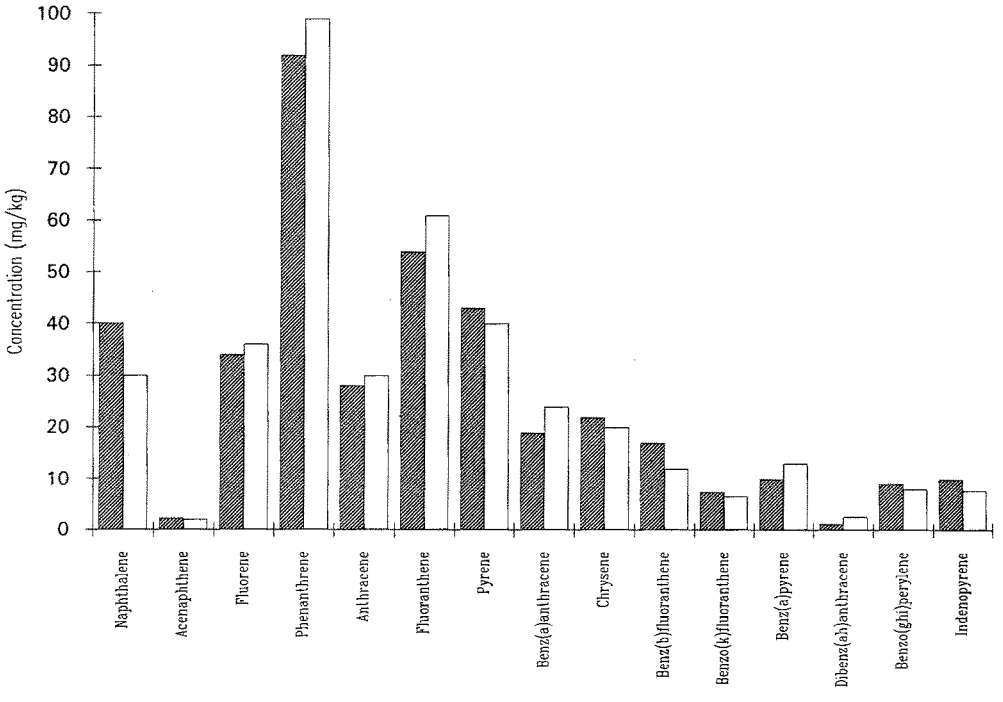

Fig. 1. PAH analysis in loam. ▨, ultrasonic extraction; □, soxhlet extraction; n = 5