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Note

Gas chromatographic determination of Lenacil and Pyrazon in soil and bacterial cultures

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The fate of herbicides in soil has been widely studied. Pyramin and venzar are commonly used as weed control agents for sugar beet and in horticulture[1,2]. Sensitive methods are required for determining trace amounts of Lenacil and Pyrazon in soil and bacterial cultures in order to provide reliable information about their behaviour under different conditions.

Spectrophotometric and thin-layer and gas chromatographic (GC) methods[3–5] have been described for the analysis of Lenacil and Pyrazon residues. Pease[4] developed a method for determining Lenacil by GC, but interpretation of the chromatograms was difficult and long retention times were obtained. No GC method for the determination of both Lenacil and Pyrazon has been reported.

The technique described have is based on the extraction of Lenacil and Pyrazon with an organic solvent, drying with magnesium sulphate, evaporation at 40 ° and GC determination using a flame-ionization detector (FID).

EXPERIMENTAL

Apparatus and reagents

A Model 18.3.6 gas chromatograph (Veb Chromatron, Berlin, G.D.R.) with a FID was equipped with a stainless-steel column (100 cm × 3 mm I.D.) packed with 3% SE-30 plus 6% OV-210 on Gas-Chrom Q (80–100 mesh) (Applied Science Labs., State College, Pa., U.S.A.). A Type 350 rotatory evaporator (Unipan, Warsaw, Poland) was used.

Venzar (commercial product containing 80% of Lenacil) and analytical-grade standard Lenacil (99.8% purity) were obtained from DuPont (Geneva, Switzerland). Pyramin (commercial product containing 80% of Pyrazon) and analytical-grade standard Pyrazon (99.8% purity) were obtained from BASF (Ludwigshaven, G.F.R.).

Chloroform, ethyl acetate and anhydrous magnesium sulphate (analytical-reagent grade) were obtained from POCh (Gliwice, Poland).

Materials

Loess soil from Biały Kościół at a depth of 5–15 cm ($C_{org} = 1.24\%$, $N = 0.12\%$, $pH_{H_2O} = 7.2$) was used. A control soil, soil containing 100 ppm of venzar, soil containing 200 ppm of pyramin and soil containing 100 ppm of venzar plus 200 ppm pyramin were prepared. All samples were enriched with 5 ml/kg of a microflora suspension from fresh garden soil and were incubated at room temperature. The water capacity of the soil was maintained at 30% (w/w).

Soil samples for analysis were taken after 4 h and 6 months of incubation; 25 g of dry soil sample were placed in a Soxhlet apparatus and extracted with chloroform for 2 h. The extracts obtained were dried with 5 g of anhydrous magnesium sulphate and evaporated to dryness at 40°.

Azotobacter vinelandii, strain A66, was cultivated in Burk's medium prepared according to Dalton and Postgate[6]. Doses of herbicides half those in the experiments with soil were introduced into the media. Medium without herbicides constituted the control sample. Herbicides were autoclaved separately in distilled water and added under sterile conditions. A 1-ml volume of 48-h-old pre-cultures of strain A66 was used as an inoculum. The experiments were carried out at 28° on a rotary shaker (60 rpm).

Post-culture media for analysis were taken after 1 h and 9 days of incubation. A 50-ml volume of sample was introduced into a separating funnel and, after adjustment of the pH to 9.0, were extracted three times with 50-ml portions of chloroform and three times with 50-ml portions of ethyl acetate. The extracts were combined, dried with 5 g of anhydrous magnesium sulphate and evaporated to dryness. All samples were examined triplicate.

Calibration graphs

Calibration graphs were prepared from standard solutions containing 0.35–5 μg of venzar and 1–10 μg of pyramin per microlitre of chloroform (see Fig. 1).

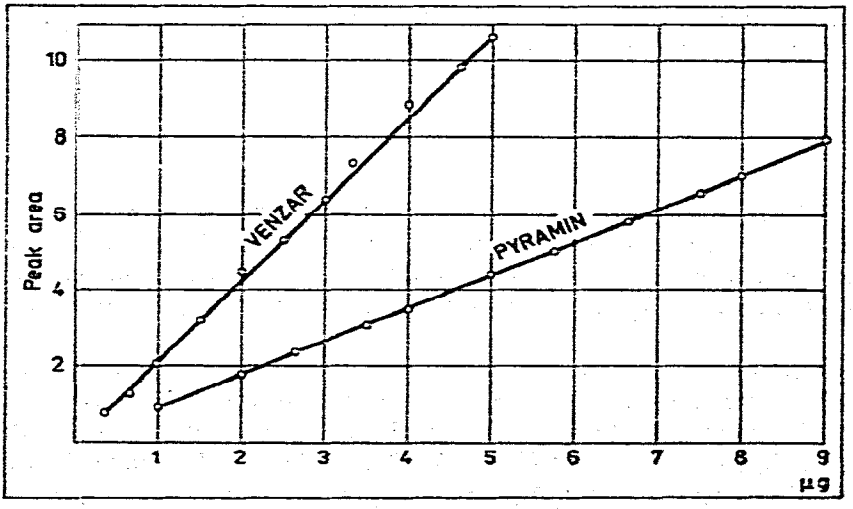


Fig. 1. Calibration graphs for pyramin and venzar.

Chromatography

The residues of the herbicides were dissoved in 1 or 2 ml of chloroform. Standard solutions and residue solutions were chromatographed using the column specified above with injector, column and detector temperatures of 295°, 230° and 310°, respectively, and nitrogen as the carrier gas at a flow-rate of 80 ml/min. Samples of 1 μl were injected. The amounts of the herbicides present were calculated from the calibration graphs.

TABLE I

GLC ANALYSIS OF SOIL TREATED WITH HERBICIDES

Time of incubation	*Herbicide*	*Sample*			
		Control	*With pyramin (200 ppm)*	*With venzar (100 ppm)*	*With pyramin (200 ppm) and venzar (100 ppm)*
4 h	Pyramin	0.0	168.0	0.0	158.0
	Venzar	0.0	0.0	96.4	96.2
6 months	Pyramin	0.0	30.5	0.0	34.5
	Venzar	0.0	0.0	32.6	70.3

TABLE II

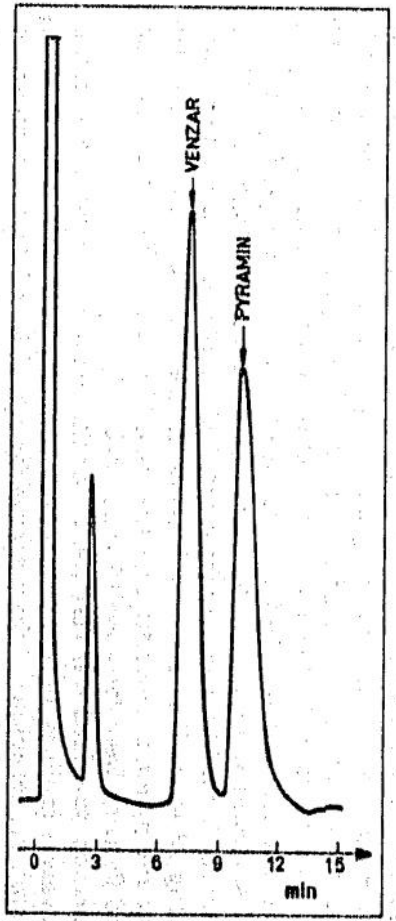
GLC ANALYSIS OF LIQUID MEDIA OF *AZOTOBACTER VINELANDII* STRAIN A66 AFTER INCUBATION

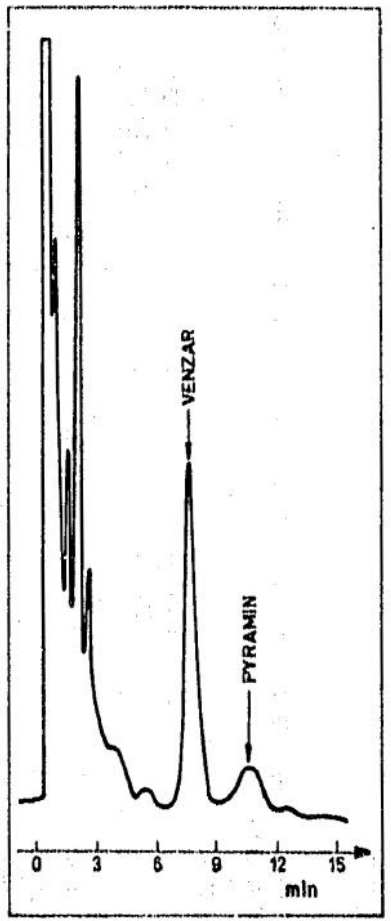
Time of incubation	*Herbicide*	*Sample*			
		Control	*With pyramin (100 ppm)*	*With venzar (50 ppm)*	*With pyramin (100 ppm) and venzar (50 ppm)*
1 h	Pyramin	0.0	96.3	0.0	94.9
	Venzar	0.0	0.0	43.5	44.5
9 days	Pyramin	0.0	94.1	0.0	92.0
	Venzar	0.0	0.0	42.2	43.1

RESULTS AND DISCUSSION

Results of studies with pyramin and venzar added to untreated soil and bacterial media after incubation are shown in Tables I and II. Recoveries obtained with this method (on the basis of the calibration graphs, Fig. 1) from soil averaged 81.0% and 96.3%, and from liquid media 95.6% and 86.0% of pyramin and venzar, respectively. The amounts of herbicides after incubation for 6 months in soil and 9 days in media with *Azotobacter vinelandii* are also given. A typical gas–liquid chromatogram of standard solutions of the investigated herbicides is shown in Fig. 2. Chromatograms of herbicides extracted from enriched samples of soil and liquid media are shown in Figs. 3 and 4. The detection limits of the method are 0.016 ppm and 0.08 ppm of venzar and pyramin, respectively, based on a 25-g sample. The retention times of Lenacil and Pyrazon were 460 and 615 sec, respectively.

The method described was very useful for the simultaneous determination of pyramin and venzar residues. In comparison with retention times and chromatograms in the literature[4], our technique is characterized by simplicity of the chromatograms,





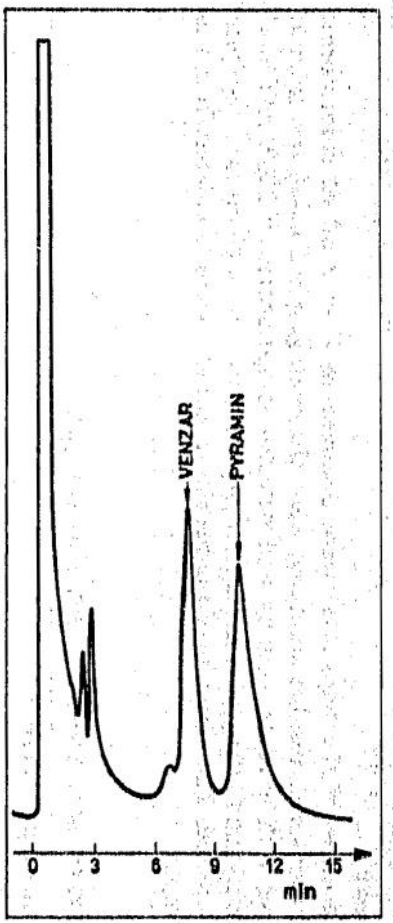


Fig. 2. Gas-liquid chromatogram of standard solution of pyramin and venzar.

Fig. 3. Gas-liquid chromatogram of pyramin and venzar extracted from soil enriched with 200 and100 ppm of the respective herbicides after incubation for 6 months.

Fig. 4. Gas-liquid chromatogram of pyramin and venzar extracted from bacterial medium enriched with 100 and 50 ppm of the respective herbicides after incubation for 9 days.

a shorter experimental time and a two-times higher sensitivity. The proposed system is suitable for routine operation. However, for other biological materials (plant tissues), it is necessary to develop a technique of purifying the sample material. These aspects are being studied.

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