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Note

Detection of triazine herbicides in soil by a Hill-reaction inhibition technique after thin-layer chromatography

JOZEF KOVÁČ and MÁRIA HENSELOVÁ
Research Institute of Agrochemical Technology 810 04 Bratislava-Predmestie (Czechoslovakia)
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Triazine herbicide residues can persist in soils in amounts sufficient to damage sensitive crops the following year and, for this reason, methods for detecting phytotoxic residue levels are often required[1,2]. The methods for the determination of residues of these herbicides in soils include bioassay[1,3,4], spectrophotometric[5–7] and chromatographic procedures[8–14]. The limits of detection of thin-layer chromatographic (TLC) methods based on chemical conversion of separated substances into derivatives suitable for visual detection range from 0.2 to 1 μg[15,16].

In this work, 2,6-dichlorophenolindophenol[17] (DCPIP) was used as an electron acceptor for the sensitive detection of Hill-reaction inhibitors on Silufol TLC plates.

EXPERIMENTAL

Materials

All reagents and solvents were of reagent grade. A buffer solution of pH 6.5 was prepared by mixing 0.06 *M* $Na_2HPO_4 \cdot 2H_2O$ and 0.06 *M* KH_2PO_4 (3:7). A solution of the sodium salt of DCPIP was prepared by heating a mixture of 40 mg of DCPIP and 100 ml of phosphate buffer (pH 6.5) on a boiling water-bath for 30 min with stirring; the solution was shaken for 30 min, filtered, cooled, diluted with distilled water to 100 ml and kept in the dark at 2 ± 1°.

Isolation of chloroplasts

Chloroplasts were isolated from leaves of 14-day-old bean plants (*Phaseolus vulgaris* L. *cv.* Harsgrus) grown in a greenhouse. A 30-g batch of leaves previously washed with deionized water was homogenized with 150 ml of 0.5 *M* sucrose solution. The homogenate was filtered through four layers of nylon cloth into a cooled flask and centrifuged at 1053 g for 10 min. The chloroplasts were re-suspended in 30 ml of 10% glycerine to give a concentration of chlorophyll of 0.29 mg/ml[18] and kept in a dark bottle at 2 ± 1°. The preparation was usually used on the same day, the maximum duration of storage being 2 days.

Stock solutions of triazine herbicides (prometryne, atrazine and simazine) in acetone were prepared in concentrations of 10, 7.5, 5.0, 2.5, 1.0 and 0.1 μg/ml.

Thin-layer chromatography

Silufol TLC plates (15 × 15 cm) were obtained from Kavalier Glass Works, Votice, Czechoslovakia. They were first developed with acetone–water (99.5:0.5), dried at room temperature for 20 min and used without activation. The developing solvent system was ethyl acetate–chloroform (1:9).

Procedure

The soil samples were air dried and sieved through an 18-mesh sieve. Samples of 50 g were extracted on a mechanical shaker with three 50-ml volumes of acetone for periods of 30 min. The extracts were filtered and concentrated to a volume of 1 ml by means of a rotatory vacuum evaporator. Mixtures of various amounts of triazine standards and concentrated soil extracts were applied to the plate using a calibrated 5-μl capillary micropipette. After development and drying at room temperature, the plates were sprayed with a mixture of chloroplast homogenate and DCPIP solution (1:2) prepared immediately before spraying. The plates were then exposed to light (three 40-W fluorescent tubes) at a distance of 20 cm until sharply defined blue-grey inhibition spots on a yellow-green background appeared. The spots so revealed were identified by comparing their R_F values with the R_F values of standard compounds (R_F values: prometryne, 0.50; atrazine, 0.35; simazine, 0.22) and quantified by visual comparison with a scale calibrated by means of known amounts of standard samples.

RESULTS AND DISCUSSION

The method permits the determination of 0.1 ppm of the tested herbicides in soils without the need for a clean-up procedure, and it is possible to determine less than nanogram amounts of the Hill-reaction inhibitors. The detection is based on inhibition of the photolytic activity of the isolated chloroplasts. The Hill reaction therefore comprises the photosynthetic electron transport for light reaction II (part of the photochemical reactions of photosynthesis[19,20]), during which water is split into hydrogen equivalents and oxygen. The reaction can be measured in *in vitro* experiments and is of particular value for the evaluation of the potency of inhibitors of photosynthesis in pI_{50} values[21,22].

The Hill-reaction inhibition technique in TLC is very rapid, sensitive and specific. It permits nanogram amounts of powerful inhibitors of photolytic activity of isolated chloroplasts to be detected, *i.e.*, about 40–45% of commercial herbicides[19]. The method can also be recommended for the semi-quantitative analysis of residues of the above herbicides in vegetables and water.

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