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A modified enzymatic detection method for thin-layer chromatograms of pesticides

Enzymatic detection of pesticides on thin-layer chromatograms has become a useful tool in analysis of these compounds. The work done by MENDOZA *et al.*[1,2], WINTERLIN *et al.*[3] and ACKERMANN[4–6] are examples of the evolution of this method. We wish to report a modification which has the advantages of simplicity, sensitivity and easy detectability for a great number of pesticides.

Materials and methods

Honey bees are caught and immediately frozen on solid carbon dioxide. The frozen bees keep their enzymic activity in a refrigerator at — 20° for at least one year. A number of bee heads (25–40) are macerated (Ultra Turrax or Waring Blendor) with 75 ml of iced water and filtered through a G1 glass filter. The "bee enzyme solution" obtained rapidly loses its activity and has to be used immediately after preparation.

A chromatogram of pesticides or crop/fruit extracts is run according to one of the known methods[1–7]. The pesticides on the chromatogram are oxidized with bromine vapour in the following way: A wide beaker with water in it is placed in a closed jar of *ca.* 25 × 25 × 25 cm. After half an hour, 0.1 ml of bromine is pipetted into the

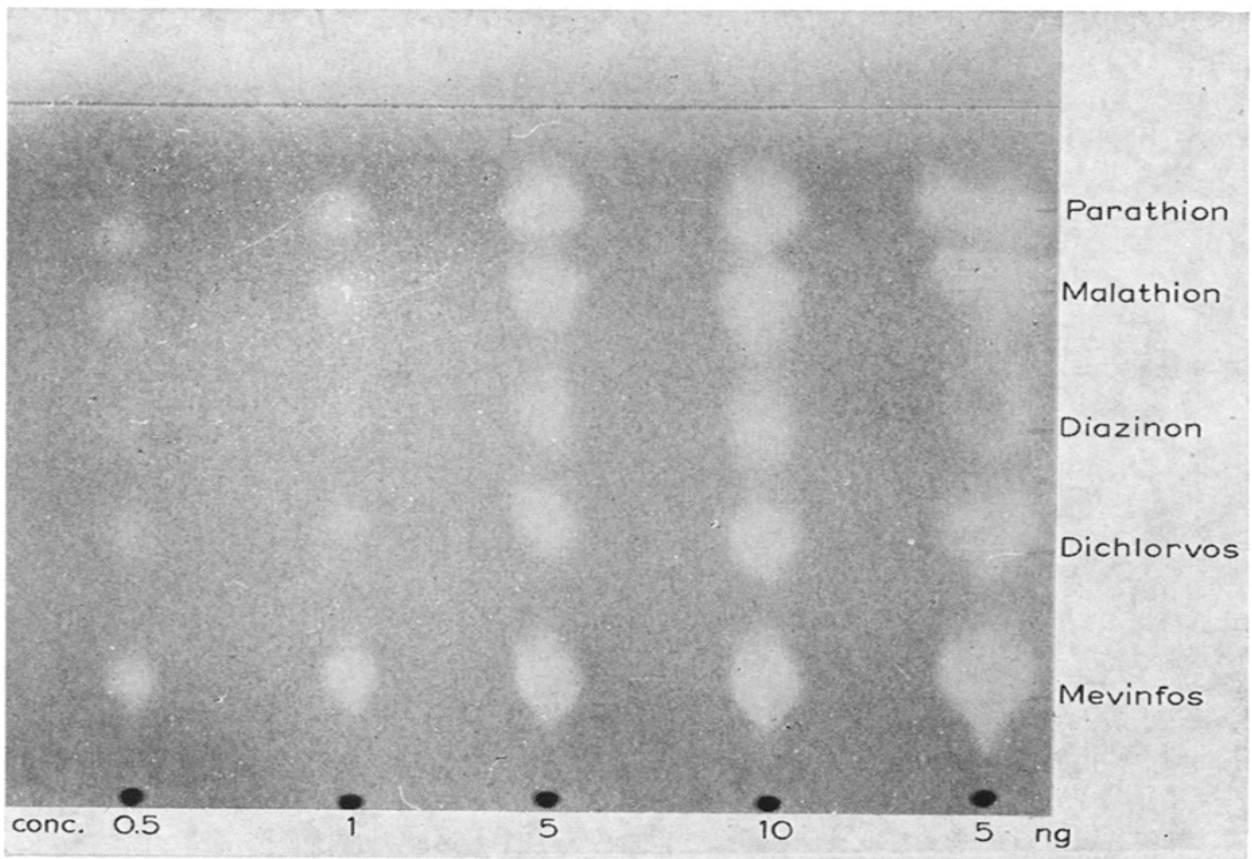

Fig. 1. Chromatogram of a mixture of five different pesticide concentrations (0.5–10 μg). Silica Gel G plate; thickness 500 μ, solvent, chloroform–ether (96:4). 5 μl of sample used except for the sample on the right hand side of the plate where 25 μl was used.

TABLE I

DETECTION OF PESTICIDES

− not detectable; ± just detectable; + detectable; ++ very easily detected.

Pesticide; common and alternative name(s)	*Chemical name*	*Sensitivity (ng)*										
		<0.1	*0.1*	*0.2*	*0.5*	*1.0*	*5.0*	*10.0*	*25.0*	*50.0*	*100.0*	*1000.0*
Azinfos-ethyl	O,O-Diethyl-S-(4-oxobenzotriazino-3-methyl) phosphorodithioate	++	++	++	++	++	++	++				
Azinfos-methyl, Guthion	O,O-Dimethyl-S-(4-oxobenzotriazino-3-methyl) phosphorodithioate	++	++	++	++	++	++	++				
Bromofos-methyl	O,O-Dimethyl-O-(2,5-dichloro-4-bromophenyl) phosphorodithioate		−	−	±	+	++	++				
Bromofos-ethyl	O,O-Diethyl-O-(2,5-dichloro-4-bromophenyl) phosphorodithioate		−	−	±	+	++	++				
Carbaryl, Sevin	N-Methyl-1-naphthyl carbamate		±	+	++	++	++	++				
Chlorphenvinfos	O,O-Diethyl-2-chloro-1-(2,4-dichlorophenyl) vinylphosphate		−	−	−	−	±	+	++			
Cidial, Phenthoat	O,O-Dimethyl-S-(1-carbethoxy-1-phenyl-methyl) phosphorothioate	±	+	++	++	++	++	++				
Demeton	O,O-Diethyl-O-(2-ethylthio)ethyl phosphorothioate					−	±	+	+	++		
Demeton-methyl	O,O-Dimethyl-O-(2-ethylthio)ethyl phosphorothioate					−	±	+	++	++		
Demeton-methyl-sulphoxide	O,O-Dimethyl-S-(2-ethylsulphinyl)ethyl phosphorothioate					−	±	+	++	++		
Diazinon	O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate		−	−	±	+	++	++				
Dichlorvos	O,O-Dimethyl-O-2,2-dichlorovinyl phosphate		−	±	+	++	++	++				
Dimethoate, Rogor	O,O-Dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate						−	−	−	−	−	+
Dioxathion, Delnax	2,3-*p*-Dioxanedithiol-S,S-bis-(O,O-diethyl-phosphorodithioate)					−	±	+	++	++		
Dithion, Dithione	O,O-Diethyl-O-7-hydroxy-3,4-teramethylene-coumarinyl phosphorothioate							−	−	−	±	+
EPN	O-Ethyl-O-(*p*-nitrophenyl)phenyl phosphorothioate	−	±	++	++	++	++	++				
Ethion, Nialate	O,O,O′O′-Tetraethyl-S,S′-methylene bis-phosphorodithioate					−	−	±	+	++	++	
Folithion, Sumithion	O,O-Dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate			−	±	+	++	++				
Hoe 2873	O,O-Diethyl-O-(6-ethoxy-carboxyl-5-methyl-pyrazolo(2,3a)pyrimidin-2-yl) phosphorothioate	−	±	++	++	++	++	++				

H 14503, Torak	O,O-Diethyl-S-(2-chloro-1-phthalimidoethyl) phosphorothioate	+	++	++	++	++	++	++				
Malathion	O,O-Dimethyl-S-(1,2-diethoxy carbamyl ethyl) phosphorodithioate		−	±	++	++	++	++				
Menazon	O,O-Dimethyl-S-(4,6-diamino-1,3,5-triazin-2-yl-methyl) phosphorodithioate		−	−	±	+	++	++				
Mevinphos, Phosdrin	O,O-Dimethyl-O-2-methoxycarbonyl-1-methylvinyl phosphate		±	+	++	++	++	++				
Nemafos, Zinophos	O,O-Diethyl-O-2-pyrazinyl phosphorothioate		±	++	++	++	++	++				
Parathion-ethyl	O,O-Diethyl-O-*p*-nitrophenyl phosphorothioate		−	±	++	++	++	++				
Parathion-methyl	O,O-Dimethyl-O-*p*-nitrophenyl phosphorothioate		−	±	++	++	++	++				
Phenkapton	O,O-Diethyl-S-(2,5-dichlorophenylthio-methyl) phosphorothiolothionate		−	−	±	+	++	++				
Phosalone	O,O-Diethyl-S-(6-chloro-2-oxo-3-benzoxazolyl-methyl) phosphorodithionate		−	−	±	+	++	++				
Phosphamidon, Dimecron	O,O-Dimethyl-O-2-chloro-2-diethylcarbamoyl-1-methylvinyl phosphate						−	−	−	−	±	+
Prothoaat, Fac 20	O,O-Diethyl-S-(N-isopropylcarbamoylmethyl) phosphorodithioate						−	−	−	−	−	±
Ronnel, Fenchlorvos	O,O-Dimethyl-O-2,4,5-trichlorophenyl phosphorothioate		−	−	±	+	++	++				
Sulfotep	O,O,O′,O′-tetraethyl diphosphorothioate						−	−	±	+	++	
Thimet, Phorate	O,O-Diethyl-S-(ethylthiomethyl) phosphorodithioate		−	−	−	−	±	+	++	++		
Thiometon	O,O-Diethyl-S-(2-ethylthio-ethyl) phosphorodithioate			−	−	−	±	+	+	++		
Trichlorphon, Dipterex	O,O-Dimethyl-(1-hydroxy-2,2,2-trichloro-ethyl phosphonate)			±	+	+	++	++				
Trithion, Carbophenothion	O,O-Diethyl-S-*p*-chlorophenylthiomethyl phosphorodithioate					−	±	+	++	++		
Ultracid, Supracid	O,O-Dimethyl-5-methoxy-(3H)-1,3,4-thiadiazol-2-onyl-(3)-methyl phosphorodithioate		±	++	++	++	++	++				
Vamidothion	O,O-Dimethyl-S-{2-1-(N-methylcarbamyl)-ethyl} phosphorothioate							−	−	−	−	+
V.C. 13 Nemacide, Dichlofenthion	O,O-Diethyl-O-2,4-dichlorophenyl phosphorothioate							−	−	−	±	+
Formothion	O,O-Dimethyl-(N-methyl-N-formyl-carbamoyl) phosphorodithioate						−	±	+	++	++	
Ortho Dibrom, Naled	O,O-Dimethyl-O-1,2-dibromo-2,2-dichloro-ethyl phosphate	±	+	++	++	++	++	++				

bottom of the tank, which is then closed to allow the bromine vapour to distribute itself evenly throughout the tank. The thin-layer chromatogram is then exposed to the bromine vapour in the tank for 30 sec, removed from the tank, and allowed to stand in air until all traces of bromine smell have vanished (30 min). The plate is next sprayed with the "bee enzyme solution" (15–20 ml for a 20 × 20 cm plate, thickness 500 μ), then kept in a moist atmosphere at 37° for half an hour. After this incubation period, the plate is sprayed again with a solution consisting of 20 mg of 2-naphthyl acetate in 8 ml of ethanol mixed immediately before spraying with a solution containing 50 mg Fast Blue B in 32 ml of water (8–10 ml of the spray reagent is sufficient for one plate, as described above). The plate is allowed to stand in a moist atmosphere at 37° for a further 15 min. The pesticides appear as white spots on a magenta coloured background.

Results

Table I shows the approximate sensitivities of the method for a number of pesticides.

Fig. 1 shows a chromatogram with different concentrations of five pesticides. The plate consists of Silica Gel G (thickness 500 μ). The solvent system is chloroform–ether (96:4, v/v). Chromatospots were made with 5 μl of liquid; the spot on the right hand side is made with 25 μl.

Remarks

The sensitivities were determined with chromatospots consisting of 10 μl of sample. Trichlorphon was isomerized into DDVP prior to detection[4]. The use of buffer solutions instead of iced water did not influence the sensitivity.

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