Effects of Some Organophosphorous Compounds and their Metabolites on Sorghum-Grain Esterase and Certain Insects Attacking Sorghum Grain

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Malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate), because of its low toxicity on mammals, has practical significance in controlling insect pests in stored grain. Rowlands (1, 2), who studied the effect of malathion and its metabolic products on esterases of wheat grain, observed no inhibition of carboxylesterase. This investigation dealt with the inhibitory effects of malathion, parathion, diazinon, diisopropylphosphorofluoridate (DFP), and their metabolic products on esterases of sorghum grain, rice weevil (Sitophilus oryzae) and granary weevil (Sitophilus granarius), and horse-serum cholinesterase.

Experimental

Organophosphorous compounds. Malathion, malaoxon, K-demethyl malathion, malathion half-ester, malathion dicarboxylic acid, Na-dimethyl phosphate, K-dimethyl phosphorothioate, K-dimethyl phosphorodithioate and paraoxon were obtained from American Cyanamid Co., Princeton, N. J. Diazinon and diazoxon were obtained from Geigy Chemical Corporation, Ardsley, N. Y. and diisopropylphosphorofluoridate (DFP) from Mann Research Laboratory, Inc., N. Y.

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The organophosphorous compounds were dissolved separately in redistilled acetone, 10 ug/ul. However, DFP was used as 1% solution in deionized water.

**Enzyme.** Cholinesterase (horse serum) CHE 6254 was obtained from Worthington Biochemical Co., Freehold, N. J. It was dissolved in 0.1 M Tris buffer pH 8.0 for use.

**Preparation and assaying sorghum grain crude homogenate.** To each 40 g portion of sorghum grain, 100 ml ice-cold 0.1 M potassium acetate buffer of pH 5.5 were added. The mixture, placed in an omnimixer container and submerged in an ice bath, was homogenized by operating the omnimixer at top speed for 1 minute. The homogenate was then centrifuged at 10,000 x g for 30 minutes and the supernatant filtered through glass wool and kept cool until used. Esterase activity was assayed by colorimetric method modified after that of Kramer and Gamson (3). A mixture of 4.6 ml 0.1 M phosphate buffer (pH 8.0) and 0.1 ml of indophenyl acetate (1 x 10^{-3}M in 95% ethanol) in an 18-mm test tube was preincubated at 30°C for 5 minutes, and organophosphorous compounds were added before the addition of 0.1 ml enzyme solution. The reaction was run at the same temperature and the absorbance was measured with a Spectronic 20 spectrophotometer at 625 mμ. Absorbance at 625 mμ was found to be proportional to the amount of enzyme in the range of 0 to 0.5 A after 10 minutes of incubation. Further incubation, up to 120 minutes, did not increase the percentage of inhibition. Organophosphorous compounds were also added to the crude homogenates (3 x 10^{-5}M/0.1 ml) and preincubated for 10 minutes at 30°C before being assayed for esterase activity. Percentage of inhibition was defined as

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\frac{A_{625} \text{ of control} - A_{625} \text{ of treated}}{A_{625} \text{ of control}} \times 100
\]

**Weevil homogenate preparation.** Weevil homogenate was prepared by grinding in a cold mortar one gram of frozen insects of the same age with about 0.5 g of 60-mesh alundum (Fisher Scientific Co., New Jersey) and 5 ml of cold 0.1 M potassium acetate buffer (pH 4.0). The homogenate was centrifuged at 10,000 x g for 30 minutes. The supernatant solution was assayed by the indophenol acetate colorimetric method as described for the sorghum-grain homogenate.

**Cholinesterase assay.** Horse-serum cholinesterase was dissolved in 0.05 M Tris buffer, pH 8.0, and assayed by the method used for the sorghum-grain esterase.