A NEW SPRAY REAGENT FOR THE IDENTIFICATION AND DETERMINATION OF QUINALPHOS, DISULFOTON AND MONOCROTOPHOS BY THIN LAYER CHROMATOGRAPHY

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QUINALPHOS, disulfoton and monocrotophos are organo phosphorus insecticides which are extensively used in agriculture for the protection of crops. These insecticides are encountered in poisoning cases in increasing number day by day. Hence it has become necessary to find a sensitive chromogenic reagent for their detection in biological material in "forensic toxicology".

Most common spray reagents are mercuric nitrate-diphenyl carbazone[1], mercurous nitrate[2], palladium chloride[3], mercuric nitrate followed by potassium ferrocyanide[4]. But it is observed that they do not react with monocrotophos and few of them which give reaction with quinalphos, are not very sensitive and hence they may escape from their detection in biological material.

In this note, a new spray reagent cupric acetate in dilute hydrochloric acid followed by potassium iodide has been described for the detection of quinalphos, monocrotophos and disulfoton on thin layer chromatographic plates. The coextracted fat from biological material did not show any interference since it did not give any coloured spot similar to the spots obtained with experimental insecticides on thin layer chromatographic plates.

The standard glass plate was coated with a slurry of Silica Gel G (ACME) with water (1:2) to a thickness of 0.25 mm. The plate was activated at 110°C for about 1 hr, 10 μg of each standard solution of quinalphos, disulfoton and monocrotophos in ethanol (1 mg/ml) were spotted on thin layer plate. The plate was then developed in a previously saturated TLC chamber using n-Hexane: Acetone (9:1) as a solvent system upto a height of 10 cm. The plate was taken out, dried in air and sprayed with (1) cupric acetate in dil. HCl (1 g of cupric acetate in 100 ml of 10% v/v HCl) followed by (2) 5% w/v aqueous potassium iodide. Different coloured spots were observed immediately on T.L.C. plate at specific R_fs. Monocrotophos gives pinkish violet coloured spot at R_f 0.58 whereas quinalphos and disulfoton give brown coloured spot at R_f 0.44 and 0.52 respectively. It is observed that some other organo phosphorus insecticides such as malathion, parathion, methyl parathion, fenitrothion, phorate, fenthion and phosalone give positive reaction with this reagent only at higher concentrations *i.e.* above 200 μg or so.

Cupric acetate reacts with potassium iodide and liberates free iodine[5]. This liberated free iodine in presence of Cu (II) in acidic medium probably react with these insecticides forming coloured complexes.

This reagent is sensitive to detect upto 10 μg of each of the above referred insecticides and hence can be used for their detection from biological material in forensic toxicology.

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IATROGENIC EFFECT OF COPPER OXYCHLORIDE SPRAYS ON *ALTERNARIA* BLIGHT OF SOME CRUCIFERS

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THE outbreak of iatrogenic diseases of plants, which result by the use of a specific crop protection chemical, is well known in the field of herbicides[1,2]. However, the records of these diseases by the use of insecticides or fungicides have been quite sporadic[2]. In the present studies, spraying with copper oxychloride was observed to increase the intensity of *Alternaria brassicae*

(Berk) Sacc infection on five cultivated crucifers. These observations were recorded in field fungicidal trials carried out during 1980–81. Results of investigations carried out on interaction of host metabolites with the copper oxychloride (blitox) are reported herein.

Five crucifers *viz.* Radish cv. Punjab safed, *Raya* cv. RL-18, Cauliflower cv. Snow ball, Cabbage cv. Local and Turnip cv. Golden ball were raised in plots, each 3 × 2.5 m in size, in the rabi season of 1981–82 and 1982–83. For knowing whether pre-infection application of blitox (50% copper oxychloride) also influenced the disease development, blitox (0.3%) and captafol (0.2%) were sprayed on healthy plants of the above crucifers 10 days prior to expected appearance of Alternaria blight. Captafol was used as it gave highly significant control of Alternaria blight on all the crucifers. To ascertain if host metabolites played any role in modyfying the toxicity of blitox, leaf exudates from these crucifers were collected by placing drops of double distilled water on the upper surface of leaves with hypodermic syringe in the laboratory for 14 hr. Exudates from each cultivar were mixed in 1 : 1 ratio with solutions of blitox and captafol for obtaining their 300 and 500 ppm concentrations respectively. Equal quantity of distilled water was added to host exudates in the case of controls. Another set of controls consisted of fungicide solutions and distilled water alone. These mixtures were incubated at room temperature (18–26°C) for 24 hr and used for spore germination assay[3] taking conidia from 12 day old cultures of *A. brassicae* on potato dextrose agar.

Like the results of previous year's trial on post-infection application of blitox, the pre-infection spraying of blitox (0.3%) also resulted in the increase of disease intensity on the test crucifers compared to untreated checks. Maximum increase in disease was recorded on radish (14.0%) and least on cauliflower (4.1%). Captafol (0.2%) provided highly significant control on all the crucifers.

Host exudates/fungicide interaction study revealed that leaf exudates of any host did not affect the toxicity of captafol and it caused cent percent inhibition of spore germination in all the cases while efficacy of blitox was reduced to varying extent by all the crucifers. Interaction with radish exudates resulted into maximum loss of toxicity of blitox which was evident from increased conidial germination (14.6%) followed closely by *raya* exudates thus giving strong indication of host/fungicide interaction (table 1). Both the rate of spore germination as well as length of germ tube was increased significantly in these interactions.

The enhancement of Alternaria blight on these five crucifers by blitox sprays, indicating its iatrogenic effect, is the first report in crop protection by the use of fungicides. However, high dilutions of copper oxychloride have earlier been reported to stimulate the spore germination in *Hemileia vastarix* causing coffee leaf rust by Nutman and Roberts[4]. They argued that residual fungicide on coffee leaves might consequently increase infection. Furtado[5] has also elucidated the effect of copper fungicides on the occurrence of pathogenic form of *Colletorichum coffeanum*. Marked decrease in the residual toxicity of Blitox on these crucifers seems to be due to strong interaction with host excretions. This effect may be the result of utilization of low concentrations of copper, left as residue on the host, by *A. brassicae* for its germination and further development on the host.

Table 1 *Influence of host leaf exudates on toxicity of blitox and captafol as observed by spore germination inhibition in A brassicae*

Fungicide (ppm)	% inhibition of spore germination in 1 1 mixture* with leaf exudates of				
	Raya	Radish	Turnip	Cauli-flower	Cabbage
Captafol (300)	100	100	100	100	100
Blitox (500)	−8.00	−14 61	−6 49	−2.36	−5 20

In fungicide solutions alone no spore germination occurred
* Pre-incubated for 24 hr at room temperature
− indicates stimulation of germination

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