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Note

Silver nitrate–bromophenol blue and potassium permanganate–sulphuric acid as chromogenic reagents for the detection of carboxin and oxycarboxin in thin-layer chromatography*

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Plant fungicides and pesticides are being used in increasing amounts in developing countries in order to increase food production. However, it is essential that the consumable parts of the plants remain free of any harmful levels of fungicides. The contamination of consumable products by fungicides is more likely to occur when fungicides are sprayed repeatedly on the parts of the plants that are used directly for consumption, such as the developing grains of cereals, vegetables and fruits. Sometimes the analysis of fungicides is also important in order to determine the amount of active ingredients present in fungicide formulations, especially those fungicides that deteriorate during storage. The analysis of fungicides from plants involves their extraction in a suitable solvent, separation and detection. Of the many methods available for separation and clean-up, thin-layer chromatography (TLC) has recently proved to be one of the easiest and most sensitive methods for the separation and identification of fungicides and other compounds. This technique has successfully been used for the analysis of dithiocarbamate fungicides such as thiram, ziram and zineb[1], substituted nitroanilines, such as 2,6-dichloro-4-nitroaniline (Botran)[2], carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) and oxycarboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido-4,4-dioxide)[3] and thiabendazole (Mertect)[4]. Fungicides such as ziram, thiram and zineb can be detected on the thin-layer plates by means of coloured spots developed by a reaction with iodine vapour[1]. Carboxin and oxycarboxin, two important systemic fungicides (Fig. 1), have so far been detected under ultraviolet light using a fluorescent indicator[3] and no suitable reagents for the visual detection of these fungicides after TLC have been available. In this paper we report two reagents, one that can detect both carboxin and oxycarboxin and the other only carboxin. These reagents make the direct detection of the two fungicides possible without the use of an ultraviolet lamp.

EXPERIMENTAL

Preparation of thin-layer plates

Thin-layer plates were prepared by coating 20 × 20 cm glass plates with 0.25 mm

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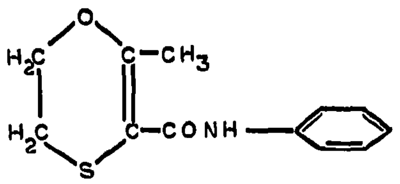
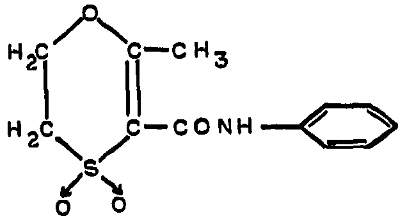


CARBOXIN (VITAVAX)



OXYCARBOXIN (PLANTVAX)

Fig. 1. Structures of carboxin and oxycarboxin.

thick layers of a silica gel suspension. The suspension was prepared by mixing 30 g of silica gel G (E. Merck, Darmstadt, G.F.R., with 13% of gypsum as a binder) with 64 ml of glass-distilled water. The presence of a few drops of methanol in the silica gel suspension greatly aided the uniform coating of the plates. The coated plates were dried at room temperature followed by oven drying at 110° for 30 min.

Fungicide solutions (10 μl) were spotted on the plates and the solvent (chloroform) was evaporated off. The plates were developed in chloroform for 90 min and then dried at room temperature and sprayed with the appropiate reagent through an atomizer.

Reagents

The silver nitrate–bromophenol blue reagent was prepared by dissolving 0.1 g of bromophenol blue in 10 ml of acetone and diluting the solution to 100 ml with a 1% (w/v) solution of silver nitrate in 1:3 aqueous acetone. The potassium permanganate–sulphuric acid reagent was prepared by dissolving 0.5 g of potassium permanganate in 15 ml of concentrated sulphuric acid.

Reaction of silver nitrate–bromophenol blue reagent with carboxin in solution

The possibility of using this reagent for the determination of carboxin in solution was investigated, and the sensitivity of the reagent in solutions of the fungicide was also studied. Volumes of 10 ml of carboxin solution were treated with 0.1 ml of reagent and the absorption spectrum of the developing coloured complex was determined using a Beckman DU spectrophotometer. The fungicide was dissolved in water because of the immiscibility of chloroform and the reagent.

RESULTS AND DISCUSSION

After spraying the plates with silver nitrate–bromophenol blue reagent, the plates were heated in an oven at 110° for 10 min. A blue colour developed with carboxin, but oxycarboxin yielded no coloured spots. The reagent not only reacted with carboxin but also with its degradation products isolated from the sprayed ears of pearl millet (*Pennisetum typhoides*), as shown in Fig. 2. Although carboxin yielded blue spots with the reagent, the silica gel itself assumed a light bluish colour. The dark blue spots of the fungicide could easily be detected even against the background colour, but this colour could be removed by spraying the plates, after spraying with the reagent, with 5% aqueous acetic acid. The plate then assumed a yellowish colour with very distinct blue spots of the fungicide. In certain instances, we found that the background colour could not be removed by the acetic acid spray, but a very brief exposure of such plates to bromine vapour bleached the background colour. In general, however, there was no necessity for removing the background colour.

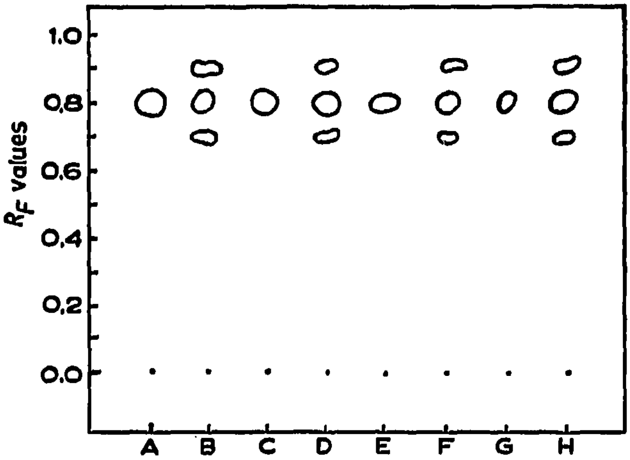


Fig. 2. Thin-layer chromatogram showing the spots of carboxin and its degradation products after detection with silver nitrate-bromophenol blue reagent. The carboxin has an R_F value of 0.8. On similar chromatograms oxycarboxin gave an R_F value of 0.4 after detection of the spots with potassium permanganate-sulphuric acid reagent. The fungicides were isolated from sprayed ears of pearl millet. A, C, E and G represent 20, 15, 10 and 2 μg, respectively, of standard, and B, D, F and H 50 μl of sample each.

The sensitivity of the reagent for carboxin was determined both on thin-layer plates and in solution. On plates, the reagent could detect down to 2 μg carboxin (Fig. 2). Aqueous fungicide solutions of different concentrations were treated with the reagent in test-tubes and the tubes were heated in hot water for 5 min. A blue colour developed which had an absorption spectrum as shown in Fig. 3, with maximum absorption at 590 nm. Studies are in progress to find a colorimetric method of determination using this method. In solution, the reagent could detect down to 5 μg/ml of carboxin.

Unlike silver nitrate–bromophenol blue, which reacted only with carboxin, the potassium permanganate–sulphuric acid reagent reacted with both fungicides. This

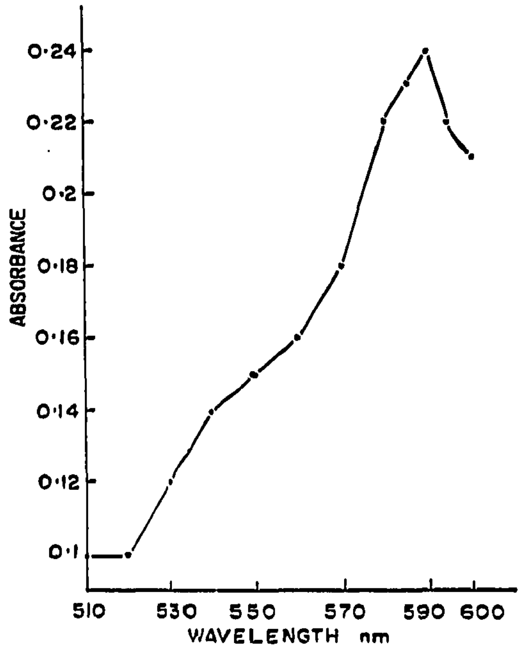


Fig. 3. Absorption spectrum of the coloured complex formed on the reaction of an aqueous solution of carboxin with silver nitrate–bromophenol blue reagent.

reagent yielded white spots of the fungicides against a pinkish background. The spots could be due to the decoloration of the permanganate by the fungicides. The lowest amount of the fungicides that this reagent could detect on thin-layer plates was 3 μg. So far we have not been able to use this reagent to detect fungicides in solution.

The R_F values given by carboxin and oxycarboxin on TLC using these reagents for detection were 0.8 and 0.4, respectively, which correspond with the values reported elsewhere[3].

The silver nitrate–bromophenol blue reagent reacts with a wide range of other fungicides. Some of the fungicides that can be detected by this reagent by TLC under the conditions reported for carboxin are thiophanate-methyl [1,2-bis(3-methoxycarbonyl-2-thioureidobenzene], RH-124 (4-*n*-butyl-1,2,4-triazole), brestanol (triphenyltin chloride), brestan (triphenyltin acetate) and benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate].

REFERENCES

1 S. C. Vyas and R. K. Tripathi, *Indian Phytopathol.*, 25 (1972) 513.
2 C. L. Keswani and D. J. Weber, *J. Chromatogr.*, 30 (1967) 130.
3 A. I. Allam and J. B. Sinclair, *Phytopathology*, 59 (1969) 1548.
4 S. M. Norman, D. C. Fouse and C. C. Craft, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 1239.