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Degradation of Carboxin and Oxycarboxin by a Species of *Pseudomonas* Isolated from Soil

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ABSTRACT

An unidentified species of *Pseudomonas* capable of utilising Carboxin and Oxycarboxin as sole source of carbon and nitrogen was isolated from red sandy loam soil perfused with solutions of these fungicides. The bacterium hydrolyzed Oxycarboxin to aniline and oxidised Carboxin to the corresponding sulphoxide and then to the sulphone. The sulphone derivative was further hydrolyzed liberating aniline. Further, degradation of aniline by the organism resulted in the accumulation of ammonium which was partly oxidized to nitrite. Nitrite accumulated in the media without further oxidation to nitrate.

INTRODUCTION

Carboxin (Vitavax: 5,6-dihydro-2-methyl-1, 4-oxathiin - 3 - carboxanilide) and Oxycarboxin (Plantvax: 5,6 dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide-4,4-dioxide) are commonly used fungicides for the control of loose smut of wheat and barley (von Schmeling and Kulka, 1966). Allam and Sinclair (1969) reported that Carboxin was metabolized to sulphone and other aniline derivatives in cotton seedlings. Chin *et al.* (1970) in an incubation study with soil were able to obtain the sulphoxide from Carboxin, but no further oxidation of the sulphoxide. Wallnofer (1969) and Wallnofer and Koniger (1972) studying the metabolism of the fungicides with *Rhizopus japonicus* reported that Carboxin was converted to the sulphoxide and then to the sulphone with accumulation of butyranilide and some other unidentified end-products. A *Nocardia*-like soil bacterium utilized Carboxin as sole source of carbon and nitrogen (Bachofer *et al.* 1973). Major metabolite of Carboxin in barley seedlings and mature plants was the p-hydroxyphenyl derivative (Briggs *et al.*, 1974). So far there are no reports on the degradation of the fungicides by microorganisms with liberation of aniline and accumulation of ammonium and nitrite.

MATERIALS AND METHODS

Red sandy loam soil (pH-6.5, organic matter 0.54 per cent) passed through 2 mm sieve was continuously perfused with the fungicides Vitavax (at 170 ppm) and Plantvax (at 1000 ppm) using a peristaltic pump with a flow rate of 0.1 ml/min for six months. From the enriched soil, a bacterium

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was isolated by serial dilution plate method (Sethunathan, 1972). The organism was identified as species of *Pseudomonas* on the basis of morphological and physiological characteristics. A 24-hr old broth culture of the bacterium grown in shake culture was centrifuged at 10,000 rpm for 10 min and washed three to four times with physiological saline (0.85 per cent) and resuspended in saline. One ml of this was inoculated into a mineral medium. The medium contained K_2HPO_4, 7.0 g; KH_2PO_4, 3.0 g; and $MgSO_4.7H_2O$, 0.1 g to a litre of distilled water; pH was adjusted to 7.0. It was dispensed in 250 ml capacity Erlenmeyer flask at the rate of 100 ml/flask. Aqueous solutions of the respective fungicides were passed through sterilized Seitz filter and added aseptically to the media to get a final concentration of 20 ppm.

No carbon or nitrogen source other than the fungicide was added to the media in the flasks. The inoculated flasks were incubated under shake culture conditions. Uninoculated media containing only fungicides served as controls. At periodical intervals one ml aliquots of culture were withdrawn and assayed for the products of degradation of the fungicides. Aniline was detected by thin-layer chromatography and also by colorimetric method (Lane, 1970). For thin layer chromatography the samples were extracted with chloroform, spotted on 0.5 mm thick silica gel plates and developed in chloroform. The plates were sprayed with 0.05 per cent fluorescein (in methanol) and the spots were detected by observing under ultra-violet light. The culture was also tested for pH and for the presence of the parent compound

Ammonium was estimated by Nesslerization, nitrite by diazotization of sulphanilic acid and coupling to N-(1 - naphthyl) - ethylenediamine hydrochloride and nitrate by 2,4-phenol disulphonic acid method (Bremner, 1965). For soil incubation studies the same red sandy loam soil from which the bacterium was isolated was used. The respective fungicides were added to the soil at three levels, namely 100, 1000 and 10,000 ppm, mixed well and placed in small plastic cups with perforated caps. The moisture content of the soil in each case was adjusted to 60 per cent of the maximum water holding capacity with sterile distilled water. The soil without the fungicide served as control. The cups were incubated at $28\pm2°C$. Moisture content was maintained constant by periodical additions of sterile water. The samples were withdrawn at monthly intervals and assayed for the products of degradation of the fungicide as in broth-culture studies.

RESULTS AND DISSCUSSION

In a synthetic liquid medium containing mineral salts and Oxycarboxin as sole source of carbon and nitrogen the bacterium was able to hydrolyze the fungicide to anilins and carboxylic acid derivative in 20 days and with fur-

ther incubation for five days both ammonia and nitrite accumulated in the medium. However, no oxidation of nitrite to nitrate was noticed.

With Carboxin as sole source of carbon and nitrogen, the bacterium oxidized Carboxin to its sulphoxide in three days and then to its sulphone in seven days. Aniline was liberated as before. Further, degradation of aniline led to the formation of ammonium and its partial conversion to nitrite. However, ammonium and nitrite did not accumulate until after forty-two days of incubation with this fungicide.

The parent compound completely disappeared from the medium with a drastic reduction of pH to 3.5 from an initial value of 7.0 on 20th day and rose to8.5 at the end of the incubation period. Formation of 5,6-dihydro-2-methyl-1,4-oxithiin-3-carboxylic acid-4,4-dioxide in the case of both Vitavax and Plantvax were detected on thin-layer chromatograms alongside aniline. The liberation of aniline was also confirmed by colorimetric estimation.

The degradation of the two fungicides was also studied in unamended red sandy loam soil. In soil-incubation studies Oxycarboxin was degraded with accumulation of ammonium and nitrite after eight months whereas, with carboxin, it took eleven months before ammonium and nitrite accumulation in the soil could be detected. With further incubation for little over 12 months, oxidation of nitrite to nitrate could not be observed. In the control flasks the parent compound of fungicides remained unaffected.

Such an extensive microbial degradation of the fungicides with the liberation of aniline, hydrolysis of aniline to ammonium and the conversion of ammonium to nitrite have not been demonstrated so far either in liquid culture or in soil-incubation studies.

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