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DEGRADATION AND PERSISTENCE OF BRESTANOL IN SUGARBEET SEEDLINGS

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ABSTRACT

Degradation and persistence of a systemic fungicide Brestanol (Triphenyl - tinchloride) known to be effective against Cercospora leaf spot of sugarbeet, was studied in sugarbeet seedlings. Thin layer chromatograms developed with 0.05% sodium fluorescein in methanol was found to be a reliable method for degradation and persistence studies. No degradation of Brestanol occurred in sugarbeet seedlings at 24 hours after treatment, however, it was converted into an entirely different compound with Rf value 0.2, 6 days after spraying. Brestanol sprayed at the rate of 450 ppm was detected at the quantity of 1.125 μg, 4 days after spraying.

INTRODUCTION

Cercospora leaf spot caused by *Cercospora beticola* Sacc. is one of the serious problems of sugarbeet (Morris and Afanasiev, 1945). Triphenyl tinchloride (Brestanol) a systemic fungicide was found to be more effective than other protectant fungicides in controlling the Cercospora leaf spot disease under field conditions (Mukhopadhyay and Rao, 1972). Solel (1971) also demostrated the systemic fungicidal activity of Triphenyl tin acetate (TPTA) and Benlate. Mukhopadhyay and Thakur (1972) for the first time demonstrated the root uptake, translocation and systemic activity of Brestanol in sugarbeet seedlings by bioassay method.

The information obtained on persistence, degradation and quantity of Brestanol present in sugarbeet seedlings after fungicidal application is reported in this paper.

MATERIALS AND METHODS

Brestanol 45 W.P. (Tri Phenyl - tin chloride (TPTC) produced by Hoechst Pharmaceuticals Limited, Bombay was used. Brestanol 450 ppm ai (active ingredient) prepared by dissolving 10 mg of the fungicide in 10 ml of acetone was used as a standard Brestanol chemical and the 10 mg of Brestanol dissolved in 10 ml of water was used as fungicide suspension for spraying sugarbeet seedlings. Sugarbeet seeds (CVM. Magnapoly) were presoaked in water overnight and 10 seeds were sown per pot of 6" size filled with soil. One month old sugarbeet seedlings were sprayed uniformly with the fungicidal suspensions to run-off with an atomizer. The leaves were harvested at 24 and 96 hours after spraying for degradation and persistence studies, respectively.

Extraction and Development of Fungicidal Spots

At the end of the required time exposure period, 8 gm leaf sample from treated plants was collected and thoroughly washed with distilled water and dried between layers of blotting papers. The cleaned leaf sample was ground in a sterile mortar and pestle and the tissues were extracted in 20 ml acetone. The 20 ml acetone fraction was concentrated to 2.5 ml in a water bath. The treated and non-treated leaf extracts and the standard fungicide at desired concentration and quantity were spotted using 10 micropipette on T.L.C. plate made of silicagel G with 13%

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gypsum at equal distance with the help of a template leaving one inch of the bottom edge of the TLC plate. Spots were kept as uniform and small as possible. The plates were developed for a distance of 10 cm in chloroform saturated solvent system then air dried and sprayed uniformly with 0.05 per-cent sodium fluorescein in methanol using an atomizer. Clear spots due to Brestanol were detectable against yellow background.

Quantitative Analysis

The standard fungicidal solution was diluted suitably to contain 0.6, 0.4, 0.3, 0.2, 0.1, 0.09, 0.05, 0.03, and 0.025 μg of fungicide per 10 μl of acetone.

Ten μl of the above standard fungicidal suspension were spotted on TLC plate for estimating the least detectable quantity. Fifty μl of the above standard fungicidal suspension was spotted on TLC plate along with 50 μl of concentrated sample solution of the leaves harvested from the treated plant at 24 hours after treatment. The diameter and colour intensity of the spots in the fungicide treated leaf extracts were compared with diameters and colour intensity of spots due to standard fungicide for the estimation of fungicide present in treated leaf extract.

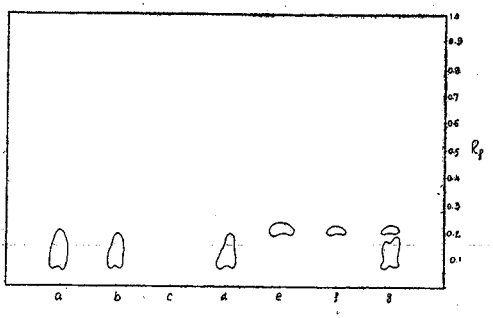
RESULTS

No spots appeared with tissue filtrates from non-treated seedlings. A single spot with brick red colour with an Rf value of 0.1 appeared due to Brestanol Standard on TLC plate against yellow background immediately after spraying the plate with 0.05% fluorescein in methanol which disappeared after drying the TLC plate. No degradation of Brestanol occurred in sugarbeet seedlings at 24 hours after treatment and the same spot appeared up to 5 days. TLC of treated leaf extract 6 days after spraying revealed a single spot with Rf value 0.2. Similar spot was continued to appear from treated leaf extract up to 28 days after spraying, but the spot obtained from tissue filtrate of 6 days after spraying interval was more intense in colour and bigger in size than the spot obtained after 28 days (Fig. 1). Brestanol could not be detected below 0.09 μg level with sodium fluorescein 0.05% in Methanol. The quantity of Brestanol present in treated leaf extract 4 days after treatment was 1.125 μg.

DISCUSSION

TLC developed with specific chromogenic reagents for the detection of spots due to Brestanol was definitely superior to bioassay technique reported by Sims *et al.* (1969) for Benlate and Erwin *et al.* (1970) for TBZ. Spots of Brestanol developed by mere spraying of 0.05% fluorescein in methanol were more clear and the UV lamp was not necessary to detect the spots. Since no spots appeared in the tissue filtrates from non treated seedlings, it can be assumed that the spots appeared in chromatographed extracts of treated plants were due to the fungicides or their degradation products. No degradation of Brestanol occurred in acetone. It was absorbed into leaf tissue in original form (Rf 0.1) and converted into an entirely different compounds with Rf value 0.2. The work carried out on Brestan (TPTA) in Germany (Personal communication from Hoechst's Pharmaceuticals Limited) suggested that TPTA was converted to Diphenyl tin acetate via various intermediates. As the Brestanol belongs to the same tin compound group, the conversion product of Brestanol may be Diphenyl tin chloride, which is comparatively stable in plant system and causes fungi-toxicity.

Results on the quantitative analysis of Brestanol, 4 days after treatment of sugarbeet leaves with 450 ppm of Brestanol showed the



DEGRADATION OF BRESTANOL IN SUGARBEET SEEDLINGS

a. Fungicide
b. Fungicide + Nontreated plant extract
c. Nontreated plant extract
d. Treated plant extract after 1, 6 and 24 days
e. Treated plant extract + Fungicide after 24 days.

presence of 7.03 ppm (TPTC), which is in agreement with the results from Hoechst's Pharmaceuticals Limited, Germany (Personal communication) on TPTA. Residue analysis of Brestanol could not be continued due to its break down into a different compound on 6th day. Break down produce of Brestanol with Rf 0.2 was detected upto 28 days after treatment in the treated sugarbeet leaves. This observation is in close agreement with the report from Hoechst's Pharmaceuticals Limited (Personal Communication) which indicates that on treating Celery plants with 360 gm. a.i./ha of Brestanol, the residue amounted to 0.03 ppm 28 days after spraying. But this 0.03 ppm is undetectable by the methods used in the present investigation. This may be the reason for not detecting the Brestanol in treated sugarbeet leaves 28 days after treatment. Thus by studying the degradation and persistence of Brestanol, it will be possible to adjust the spray concentration and to prolong the intervals between successive spray treatments for allowing liberal time schedule.

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