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Effect of Certain Fungicides on the *in vitro* Growth and Enzymatic Activities of *Acrocylindrium oryzae* Sawada

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ABSTRACT

The growth of the rice sheath rot fungus, *Acrocylindrium oryzae* was effectively inhibited by Benlate and Hinosan at 0.005 and 0.05 per cent respectively. Maneb and Zineb were inhibitory only at higher concentrations. Quinalphos, an insecticide, inhibited the growth of the fungus at 0.4 per cent. The activity of cellulase was completely inhibited by quinalphos and moderately by Benlate and Hinosan. Maneb and Hinosan were highly inhibitory to amylase activity. The activity of pectin *trans* eliminase was supressed by Hinosan while polygalacturonate *trans* eliminase activity was effectively inhibited by all the chemicals tested. Maneb and quinalphos were highly inhibitory to peroxidase activity.

INTRODUCTION

Fungicides are known to inhibit the metabolism of several fungal pathogens (Nene, 1971). They react mainly with the susceptible enzymes containing SH groups and also inactivate other enzymes like dehydrogenases, coenzyme-A, polyphenol oxidase etc. (Owens, 1963). Many fungicides also inhibit the citrate synthesis from acetate and respiration of several fungi (Owens and Novotny, 1959). The present paper reports about the effect of certain fungicides on the *in vitro* growth and activities of various enzymes elaborated by *Acrocylindrium oryzae*, the sheath rot pathogen of rice.

MATERIALS AND METHODS

For in vitro growth : To 50 ml of the Czapek's broth in 250 ml Erlen Meyer flask different concentrations of the test chemicals *viz.* Benlate (Methyl-1- (butyl carbamoyl) 2-benzimidazole-carbamate), Hinosan (0-ethyl-S, S-di-phenyl-phosphorodithioate), Zineb (zinc ethylene bisdithiocarbamate), Maneb (manganese ethylene-bisdithiocarba-mate) and quinalphos (0, 0-diethyl-0 (quinoxalinyl)-(2) - thionophosphate), dissolved in small quantities of ethyl alcohol added after sterilizing the medium. The medium was inoculated with 4 mm disc of 10 days old culture of *A. oryzae* grown on Czapek's medium and incubated at 28°C±2°C for 15 days. The mycelial mat was removed and filtered through previously dried Whatman No. 41 filter paper, dried at 105°C for 25 hr and weighed till constant weight was obtained.

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For enzyme assays: Czapek's medium devoid of sucrose was supplemented with 1 per cent either cellulose or pectin as carbon source. In another set 50 mg of *p*-hydroxybenzoic acid was added to the Czapek's medium for assaying peroxidase and polyphenol oxidase activities. The medium was sterilized and inoculated as described early. At the end of the incubation period the culture filtrate obtained from pectin-Czapek's medium was dialysed against large volumes of distilled water for 16 hr at 8°C and used for assaying pectin *trans* eliminase (PTE) and polygalacturonate *trans* eliminase (PGTE). While for assaying cellulase (Cx), amylase, peroxidase and polyphenol oxidase the culture filtrates were used as enzyme sources.

Cellulase (Cx), PTE and PGTE were estimated following the methods detailed by Mahadevan (1975). Amylase (Nelson, 1944), peroxidase (Hampton 1963) and polyphenol oxidase (Matta and Dimond, 1973) were analysed by the standard methods. To ascertain the effect of the test chemicals on the activities of various enzymes, different concentrations of the chemicals dissolved in the appropriate buffers or dist. water were used in the assay mixture in the place of the buffer or dist. water.

RESULTS AND DISCUSSION

The present study indicates that all the test chemicals supressed the *in vitro* growth of *Acrocylindrium oryzae* at various concentrations. Quinalphos

TABLE. Effect of fungicides on the growth and emzyme activity of *Acrocylindrium oryzae*

Fungicides	Concentration %	Mycelial dry weight mg / 50 ml	Cx activity (mg of reducing sugars released/ml of enzyme)	Amylase activity (mg of reducing sugars released/ml of enzyme)	Pectin trans-eliminase (% loss in viscosity of pectin)	Polygalacturonate trans eliminase (% loss in viscosity of sodium polypectate)	Peroxidase activity in units
Control	—	642	76	83	17	60	83
Benlate	0.005	0	38	95	15	0	63
	0.01	0	23	76	15	0	—
	0.05	0	—	0	15	0	73
Dinosan	0.1	0	47	0	0	0	—
	0.1	0	—	0	15	0	0
Maneb	0.2	0	61	0	14	0	—
	0.1	0	—	144	11	0	83
Zineb	0.2	0	76	79	10	0	—
Quinalphos	0.1	63	42	122	—	0	0
	0.2	40	0	84	17	0	—
	0.4	0	—	69	15	0	—

— Not tested

an insecticide exerted complete inhibition on its growth at 0.4 per cent (Table). Adaickalam (1974) reported that *in vitro* growth of *Helminthosporium oryzae* was completely inhibited by Hinosan, moderately by Maneb, Zineb and Benlate at 0.04 per cent concentration.

Toxicity of the these chemicals against the *in vitro* growth of fungi may be attributed to their ability to inhibit the general metabolism especially the enzymes of the pathogens. Ragunathan (1974) reported that *A.oryzae*, elaborated large amounts of cellulase (Cx), amylase, PTE and PGTE *in vitro* and indicated the possible involvement of these enzymes in sheath rot syndrome. In the present study, the Cx activity of the fungus was completely inhibited by qninalphos and moderately by Benlate and Hinosan. Maneb exerted less inhibition while Zineb was ineffective on the Cx activity (Table).

The amylase activity of *A. Oryzae* was strongly inhibited by Hinosan and Maneb even at low concentrations but moderately by other fungicides. However, with increase in concentration of Benlate,Zineb and quinalphos, increased inhibition was observed. The present study also reveals that the activities of PTE was completely arrested by Hinosan at higher concentration (0.1 per cent) but low concentration (0.05 per cent) was least inhibitory. Benlate, Maneb and quinalphos were less toxic to the PTE activity even at higher concentration. But all the test chemicals were found to be highly inhibitory to the PGTE activity even at low concentration. Peroxidase activity of *A. oryzae* was completely inhibited by Maneb and quinalphos at 0.1 per cent while other fungicides were least inhibitory to the enzyme activity (Table). Since polyphenol oxidase was feebly elaborated by the pathogen, inhibition of the enzyme could not be detected in the present study.

Our experiments clearly indicate that the chemicals tested against *A. oryzae* exhibited different modes of action on different enzymes of the pathogen and inhibition of these enzymes may possibly inactivate the pathogen during pathogenesis. Further quinalphos which is recommended as an insecticide may also have some role against sheath rot disease of rice.

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