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Plant Pathology

EFFECT OF FUNGICIDES ON THE LIPASE INHIBITION IN FUNGI

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The effect of eight commonly recommended fungicides, aureofungin (antibiotic) lunasan, agrosan GN, plantvax, vitavax, thiram, captan and beet powder were studied on 19 most dominant fungi isolated from oil-seeds, oil-cakes and other oil preparations. All the fungicides showed complete inhibition of fungal growth and lipase secretion at 150 μg/ml concentration, whereas, doses below 100 μg/ml were not effective in all cases except agrosan GN and aureofungin. Fungi also showed variable behaviour in enzyme secretion and dose response.

INTRODUCTION

Lipase is the trivial name of the fat-hydrolyzing enzyme and it was first of all reported by Gerrard (1897) to be secreted by *Penicillium glaucum*. Later, it was confirmed that large number of fungi secrete lipase to bring about the hydrolysis (lipolysis) of fats and oils releasing free fatty acids and glycerol (lipoclasis). The enzyme is secreted extracellularly by the fungi, bacteria and actinomycetes in the substrate in which they grow. Workers have been trying since long to protect fatty substrates from the effect of microbial lipase during storage of these products. Large number of fungicides and inhibitors are suggested for this purpose (Mc Callan, 1967; Torgeson, 1967), but all are not equally effective against all types of fungi. In the present paper eight commonly recommended fungicides are assayed against 19 most dominant and lipolytic fungi isolated from stored sesamum, cotton, castor seeds their oil cakes and other preparations. These studies will enable to find out the relative lipase secretion capacity of various fungi and their growth on the fatty substances.

MATERIALS AND METHODS

The 8 common fungicides i.e., agrosan GN (1% phenylmercury acetate and ethylmercury chloride), thiram (75% tetramethyl thiramdisulphide), captan-*N* (trichloromethylthio-4-cyclohexane, 12 dicarboxymide), vitavax (2–3 dihydro, 5-carboxynilide, 6 methyl 1–4 oxathin, 10% dust) plantvax (2–3 dihydroxyinilido, 6 methyl 1–4 oxathin-4 dioxide, 5% granular 5% WP) beet powder, lunasan, and aureofungin (heptane antibiotic from *Streptomyces cinnamomeus* var. *terricola* the aeromatic moiety is *N*-methylpamino-acetophenone and mycosamine) were selected and their effect was determined in relation to lipase production by Agar Plate method. Rahn's (1905) and Czapek's Dox media were prepared and 5% emulsified olive, sesamum, castor and cotton seed oils

were added. The nile-blue sulphate as an indicator was also mixed in 1.5 × 10^{-4}/ml concentration of the medium (Turner, 1927). After sterilization of the media the fungicidal solutions were added in each cooled medium in calculated amount so as to reach their concentration 50, 100 and 150 μg/ml of the medium separately. The plates prepared from these media were inoculated with pure spore suspension of the test fungi, incubated at 28°C ± 1°C and the observations were taken after 7 days.

Results and Discussion

The results were observed on the basis of the colour change from red to blue in the medium. The unhydrolysed fat globules in the medium are stained red in colour by the nile blue sulphate, but after the growth of organism the red globules become blue in colour. This indicates hydrolytic action of lipase on fat globules in the medium due to release of free fatty acids and secretion of lipase in presence of fungicide in substrate. The results are included in Table I. In general, four categories were recognised:

(i) After 7 days incubation period at 28°C (± 1°C) some of the micro-organisms grew well on media surface and showed the secretion of lipase within the duration required for the emergence of colony.

(ii) Some of the organisms showed usual colony growth but the reaction for lipase production appeared quite late (moderate lipolysis).

(iii) Many fungi showed a slow growth and very slow colour reaction.

(iv) Rest of the organisms exhibited very poor growth and no lipase secretion in comparison to control.

A. niger, A. flavus, A. tamarii, A. nidulans and *Trichothecium roseum* could grow in 50 μg/ml concentration of aureofungin while *A. fumigatus, Penicillium chrysogenum, P. lilacinum* and *Mycelia sterelia* could grow in this concentration but lipase secretion was completely suppressed. *A. sydowi, A. sulphureus* and *P. citrinum* showed slow lipoclasis (III category). *A. niger* grew at 100 μg/ml concentration with moderate lipase secretion while *A. sydowi, P. citrinum* and *P. chrysogenum* did not show the reaction of lipase production. These observations indicated that the suppression of lipase production in the medium was caused by the action of fungicide. Variable concentrations of such chemicals are needed for the checking of enzymatic activity and growth. In lunasan, 15 species could grow at 50 μg/ml. Out of these, 10 species showed fast lipolysis and others showed slow reaction. *A. tamarii* and *P. chrysogenum* exhibited fast reaction while others were quite slow at 100 μg/ml. Only 6 species grew at this concentration and 6 in 150 μg/ml of lunasan. Similar behaviour was exhibited by plantvax, vitavax, beet powder but none of the species could grow at 150 μg/ml concentration of the fungicides. In plantvax, vitavax, thiram and beet powder 5, 6, 3 and 5 species could grow respectively at 100 μg/ml with slow lipase production. Agrosan-GN and captan showed effective suppression of lipase production. Only 4 species grew at 50 μg/ml concentration of agrosan— 2 species showed moderate, 1 specie was slow and the other showed negative reaction.

It is clear from these studies, that fungicides could suppress the lipase secretion by micro-organisms and were found effective in variable concentrations. A concentration of 100 μg/ml of aureofungin, lunasan, plantvax, vitavax, thiram, captan and beet powder controlled lipase secretion in fungi effectively. These studies indicated that

Effect of fungicides on growth and production in Fungi

Microflora	Fungicides (μg/ml)																								
	Aureofungin			Lunasan			Agrosan GN			Plantvax			Vitavax			Thiram			Captan			Beet powder			Conrol
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	
Aspergillus niger	G‡	G*	—	G‡	G*	G*	G†	—	—	G‡	G‡	—	G‡	G‡	—	G†	G*	—	G‡	G*	—	G‡	G*	—	G‡
A. flavus	G‡	—	—	G‡	G†	G*	G†	—	—	G†	G*	—	G‡	G*	—	G‡	G†	—	G‡	G†	—	G‡	G‡	—	G‡
A. sydowi	G*	—	—	G*	G*	G*	—	—	—	—	—	—	G*	G*	—	—	—	—	—	—	—	—	—	—	G‡
A. tamarii	G‡	—	—	G‡	G‡	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G‡	G*	—	G‡
A. fumigatus	G§	—	—	G‡	—	—	—	—	—	—	—	—	G†	G*	—	—	—	—	—	—	—	G*	G*	—	G‡
A. terreus	G‡	—	—	G*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G‡	G§	—	G‡
A. nidulans	G‡	—	—	G‡	—	—	—	—	—	G‡	G*	—	—	—	—	—	—	—	G*	—	—	G‡	—	—	G‡
A. sulphureus	G*	—	—	—	—	—	G†	—	—	G‡	—	—	G†	—	—	—	—	—	G*	—	—	—	—	—	G‡
Penicillium citrinu'n	G*	G*	—	G*	—	—	—	—	—	—	—	—	G†	G*	—	—	—	—	—	—	—	—	—	—	G‡
P. chrysogenum	G§	G§	—	G‡	G‡	G*	—	—	—	G†	—	—	G§	—	—	—	—	—	—	—	—	G‡	—	—	G‡
P. lilacinum	G§	—	—	G*	—	G*	G§	—	—	G‡	—	—	—	—	—	G‡	—	—	—	—	—	G‡	—	—	G‡
Spicaria sp.	—	—	—	G‡	—	—	—	—	—	G†	—	—	G*	G*	—	—	—	—	—	—	—	—	—	—	G‡
Fusarium moniliforme	—	—	—	G‡	—	—	—	—	—	—	—	—	G*	—	—	G‡	—	—	—	—	—	—	—	—	G‡
Cladosporium herbarum	—	—	—	G*	—	—	—	—	—	G†	G*	—	G*	—	—	—	—	—	G†	—	—	G*	—	—	G‡
Cephalosporium sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G*	—	—	G‡
Syncephalastrum racemosus	—	—	—	—	—	—	—	—	—	—	—	—	G†	—	—	—	—	—	—	—	—	—	—	—	G‡
Rhizopus stolonifer	—	—	—	—	—	—	—	—	—	G*	G§	—	G*	—	—	G*	—	—	G*	—	—	G*	—	—	G‡
Mycelia sterilea	G§	—	—	G‡	G*	G§	—	—	—	—	—	—	G*	—	—	G*	—	—	—	—	—	G*	—	—	G‡
Trichothecium roseum	G‡	—	—	G‡	—	—	—	—	—	G†	—	—	G	—	—	G‡	G*	—	—	—	—	—	—	—	G‡

G, Growth of organism; ‡, Strong positive reaction; †, Moderate positive reaction; *, Slow positive reaction; § Complete inhibition of Enzyme & Growth.

fungicides not only affect the growth of organisms but also interfere with the process of enzyme production. Grossman (1962) made similar observations in *Fusarium* wilt in tomato and studied the effect of rufianic acid on proteolytic enzymes of *F. oxysporum*, and *F. lycopersici*. Burchfield (1967). emphasized that effectiveness of fungicides should be evaluated on the basis of their biological activity because they function by disrupting cell membrane through surface effect and precipitating enzymes and other macromolecules or reacting indiscriminately with amino acids, peptides and other intermediate metabolites.

Therefore, it can be concluded that irrespective of the growth of fungi on the oileagenous seeds or substrate, their lipase secretion can be checked by use of such chemicals in lower concentration to check the biodeterioration.

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