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Determination of Antifungal Activity of Some Organic Chemicals

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Abstract. Antifungal activity of four organic compounds was determined against fungi. Out of these *m*-chloronitrobenzene and 2, 3-dichloro-5, 6-dicyno- 1, 4-benzoquinone (DDQ) were found most effective at 250 ppm (0.025%), while *p*-chloroanaline and *p*-bromobenzene gave satisfactory results. In *in vivo* testing DDQ showed best results at 0.50 per cent concentration in fat liquor.

1. Introduction

Various kinds of chemicals have been used for several decades to check the growth of micro-organisms on different materials. The constant use of such antimicrobial compounds induce resistance in micro-organisms against their toxic effect. Therefore, it is quite essential to test newer chemicals and their better formulations, which may impart persistent antimicrobial effect to the material for a longer duration, to check the process of biodeterioration. Similar attempts have been made in the present investigations.

2. Material and Methods

The laboratory evaluations to find out the inhibitory concentration was made following the poisoned food technique[1]. The calculated amount of chemicals was incorporated in sterilized Czapecks Dox agar medium to obtain the desired concentration. The medium was distributed in sterilized petridishes which were inoculated aseptically with a drop of spore suspension of various test fungi. The petridishes were incubated at 28 $\pm$ 1°C temperature. The diameter of the colony was measured after seven days.

The *in vivo* testing of these chemicals to find out the suitability and working concentration, was performed at Government Leather Institute, Nunihai, Agra. This

was done taking 6″ × 6″ pieces of tanned cow hide. The leather pieces were immersed in fat liquor emulsion, containing variable concentrations of chemicals on weight basis, for four hours. After processing, the pieces were taken out and subjected to tropical chamber test[2] to see the mould growth. The mould resistance test was also performed by leaching the fungicide incorporated leather pieces, following the procedure given by American Association of Leather Chemists[3]. The incorporation of these chemicals in tanning liquor was not possible because of their non-solubility in tanning solution.

3. Results

As indicated by the results shown in Table 1, all four chemicals were effective at 250 ppm (0.025%) concentration. Among these, *m*-chloronitrobenzene and 2, 3-dichloro-5, 6-dicyno-1, 4 benzoquinone (DDQ) were most effective against all the organisms. All the fungi were inhibited 100 per cent by *m*-chloronitrobenzene at 250 ppm (0.025%) except *Curvularia lunata* and *C. senegelensis*, which were reduced to 0.9 and 0.6 cm. in comparison to their respective controls. DDQ inhibited growth of all organisms at 250 ppm (0.025%) except *Mucor* sp. *Aspergillus tamarii, A. awamorii and Mucor* sp. showed growth at 200 ppm (0.020%) concentration of DDQ, while all others were inhibited. The 50 ppm (0.005%) concentration of DDQ inhibited the 100 per cent growth of *A. niger, A. flavus, A. sulphureus, A. candidus, A. ochraceous, A. sydowi, A. sydowi* var. *agraii, A. luchuensis, A. chevalieri, A. amstelodami, Penicillium citrinum, P. cyanum, P. variable, P. purpurogenum, P. simplicissimum, P. fellutanum, Curvularia lunata, C. sinegelensis, Alternaria alternata, A. tennuissima, Fusarium solani* and *Mucor* sp.

p-chloroanaline and *p*-bromobenzophenone were also effective at 250 ppm (0.025%) concentration. At 250 ppm of *p*-chloroanaline, *A. flavus* (scl.), *Paecilomyces variotii, Drechselera papendorfii, Fusarium solani, and Caphalosporium* sp. showed little growth while, others were completely inhibited. *Aspergillus niger, A. flavus, A. flavus* (Scl.) and *Curvularia lunata* showed growth at 250 ppm (0.025%) concentration of *p*-bromobenzophenone.

The *in vitro* testing was carried out in fat liquor emulsion (emulsified fish and Turkey red oil in water) at 0.025, 0.050 and 0.10 per cent concentrations respectively and the results are shown in Table 2. The appearance of different fungi on chemicals incorporated leather pieces was noted after 30 and 60 days respectively. Out of four chemicals, DDQ completely checked the growth of all test fungi at 0.50 per cent concentration, while rest of these could show this behaviour at higher concentration. The tests for resistance by leaching process also confirmed the efficacy of these chemicals. The persistence of these chemicals against the leaching effect of water was also confirmed as shown for Table 2.

Table 1. Effect of chemicals on the growth of fungi. (Fungal growth in cm.)

Fungi	Control	Chemicals																			
		p-chloroanaline					*p*-bromobenzophenone					*m*-chloronitrobenzene					DDQ				
		Concentration					Concentration					Concentration					Concentration				
		50	100	150	200	250	50	100	150	200	250	50	100	150	200	250	50	100	150	200	250
Aspergillus niger	2.8	2.5	2.0	1.4	—	—	2.8	2.3	1.9	1.5	0.8	2.4	1.9	1.2	0.8	—	1.8	0.5	—	—	—
A. flavus	3.2	2.6	2.1	1.2	0.2	—	3.0	2.7	2.3	1.8	1.4	2.5	1.6	0.5	—	—	—	—	—	—	—
A. flavus (scl.)	3.2	2.7	2.4	2.0	1.2	0.4	2.8	2.5	2.1	1.7	1.3	2.4	1.1	—	—	—	—	—	—	—	—
A. fumigatus	3.5	2.4	1.0	—	—	—	2.1	1.1	0.2	—	—	1.7	0.5	—	—	—	2.5	1.4	—	—	—
A. terreus	2.6	1.2	0.4	—	—	—	2.0	1.2	0.4	—	—	0.8	—	—	—	—	1.0	0.2	—	—	—
A. tamarii	2.9	2.0	1.1	0.6	—	—	1.5	0.6	—	—	—	1.6	0.6	—	—	—	2.0	1.6	1.0	0.4	—
A. sulphureus	1.5	—	—	—	—	—	0.4	—	—	—	—	0.6	—	—	—	—	—	—	—	—	—
A. candidus	1.7	0.8	—	—	—	—	0.9	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—
A. ochraceous	1.7	1.0	0.4	—	—	—	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A. sydowi	1.4	0.8	0.3	—	—	—	0.8	0.3	—	—	—	—	—	—	—	—	—	—	—	—	—
A. sydowi var. *agarii*	1.6	0.5	—	—	—	—	0.9	0.4	—	—	—	—	—	—	—	—	—	—	—	—	—
A. nidulans	1.5	1.0	0.4	—	—	—	1.0	0.2	—	—	—	1.5	1.0	0.6	0.2	—	—	—	—	—	—
A. luchuensis	1.6	1.2	0.9	0.2	—	—	1.2	0.8	0.3	—	—	—	—	—	—	—	—	—	—	—	—
A. japonicus	2.5	2.0	1.2	—	—	—	1.8	1.0	0.2	—	—	2.0	1.4	0.9	0.2	—	2.0	1.4	0.6	—	—
A. awamorii	1.8	1.2	0.5	0.2	—	—	1.2	0.6	—	—	—	—	—	—	—	—	1.8	1.2	0.5	0.2	—
A. chevalieri	1.5	1.1	0.4	—	—	—	1.1	0.6	0.2	—	—	—	—	—	—	—	—	—	—	—	—
A. amstelodami	2.0	1.5	0.5	—	—	—	1.2	0.8	—	—	—	1.1	0.2	—	—	—	—	—	—	—	—
Paecilomyces varioti	2.52	.0	1.8	1.5	1.0	0.8	1.8	1.0	0.2	—	—	1.2	0.4	—	—	—	1.8	1.0	0.3	—	—
Cladosporium cladosporoides	1.1	0.4	—	—	—	—	0.9	0.3	—	—	—	1.1	0.9	0.4	—	—	1.0	0.4	—	—	—
Chaetomium globosum	1.8	1.4	1.0	0.2	—	—	1.2	0.8	0.2	—	—	—	—	—	—	—	0.5	—	—	—	—

(Contd.)

(Table 1. Contd.)

Penicillium citrinum	1.4	0.5	—	—	—	—	0.8	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—
P. cyaneum	1.2	—	—	—	—	—	0.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
P. oxalicum	2.2	1.6	0.5	—	—	—	1.8	1.5	0.7	—	—	1.5	0.8	—	—	—	1.6	0.5	—	—	—
P. variable	2.0	1.2	0.7	—	—	—	1.1	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—
P. purpurogenum	1.7	1.5	1.0	0.4	—	—	1.2	0.8	—	—	—	—	—	—	—	—	—	—	—	—	—
P. funiculosum	2.9	2.2	1.4	0.8	0.2	—	2.0	1.1	0.5	—	—	2.0	1.1	0.5	—	—	1.2	0.6	—	—	—
P. expensum	2.5	1.8	1.0	0.3	—	—	2.0	1.6	1.2	0.4	—	1.6	0.8	0.2	—	—	1.5	0.2	0.2	—	—
P. simplicissimum	1.8	1.2	0.4	—	—	—	1.2	0.7	0.2	—	—	1.0	—	—	—	—	—	—	—	—	—
P. fellutanum	1.9	1.4	0.9	0.3	—	—	1.3	0.8	0.2	—	—	—	—	—	—	—	—	—	—	—	—
Curvularia lunata	2.0	1.5	0.8	0.4	—	—	1.6	1.1	0.9	0.6	0.2	2.0	2.0	1.8	1.5	0.9	—	—	—	—	—
C. senegelensis	1.5	0.8	—	—	—	—	1.0	0.4	—	—	—	1.5	1.5	1.5	0.8	0.6	—	—	—	—	—
Trichoderma harzianum	1.7	1.1	0.5	—	—	—	1.3	0.9	0.3	—	—	1.0	0.4	—	—	—	0.5	—	—	—	—
T. viride	1.4	0.7	—	—	—	—	0.8	0.2	—	—	—	—	—	—	—	—	0.8	0.3	—	—	—
Alternaria alternata	2.2	1.8	1.2	0.7	—	—	1.7	1.0	0.5	0.2	—	1.0	—	—	—	—	—	—	—	—	—
A. tenuissimma	1.5	1.0	0.4	—	—	—	1.4	1.1	0.6	0.2	—	—	—	—	—	—	—	—	—	—	—
Drechslera papendorfii	1.8	1.6	1.3	0.9	0.6	0.4	1.1	0.5	0.2	—	—	1.4	0.7	0.3	—	—	1.8	1.0	0.4	—	—
Fusarium solani	2.0	2.0	1.6	1.2	0.9	0.6	1.5	1.1	0.8	0.6	—	1.1	0.5	—	—	—	—	—	—	—	—
Mucor sp.	2.5	—	—	—	—	—	2.0	0.4	—	—	—	—	2.0	1.2	0.5	—	2.1	1.5	1.0	0.6	0.2
Rhizopus sp.	2.8	—	—	—	—	—	2.1	1.0	0.3	—	—	—	—	—	—	—	—	—	—	—	—
Cephalosporium sp.	2.6	2.4	2.0	1.9	1.6	1.2	1.9	1.2	0.5	—	—	0.4	—	—	—	—	1.2	0.3	—	—	—

Table 2. In vivo Testing of Chemicals

Chemicals	Concentration *w/w*											
	6	0.025%			0.050%				0.100%			
Days	30		60		30		60		30		60	
	UL	L	UL	L	UL	L	UL	L	UL	L	UL	L
p-chloroanaline	9,3	9,3,2	3,9,6 1,7	3,9, 6,1, 7	3	3,6,2	3,9, 4	—	—	—	—	—
p-bromobenzo-phenone	1,9	1,9	1,2,3, 5,9	1,2,3 5,6,2	1,2	1,2	1,2,3, 9,4	—	—	—	—	1,2
m-chloronitro-benzene	5,6	5,2,6	5,6,3, 9,4	5,6,1, 2,3,4, 8,7,9	6,3	6,3	6,3,2, 1,7,5	—	—	—	—	—
DDQ	—	2,6	8,9	9,2,1	—	—	—	—	—	—	—	—

UL = unleached leather pieces; L = leached leather pieces

Fungi :

Indicated by numbers

1. *Aspergillus niger*
2. *A. flavus*
3. *A. flavus* (scl.)
4. *Cepholosporium* sp.
5 *Curvularia lunata*
6. *C. senegelensis*
7. *Drechselera papendorfii*
8. *Fusarium solani*
9. *Paecilomyces variotii*

4. Discussion

It is evident from the observations that *m*-chloronitrobenzene and DDQ were found very much effective at 250 ppm concentration in laboratory testing while higher concentration was required for inhibition of fungi in *in vivo* testing. DDQ was best as it gave complete protection at 0.050 per cent while *m*-chloronitrobenzene gave similar results at 0.10 per cent concentration.

Such studies have also been conducted earlier by different workers who have tested different kinds of compounds viz., 4-nitromethyl esters including mixed carbamates and bicarbamates and 5, 6-dichloro-2-benzoxazolinone [4,5]. *B*-naphthol[6], copper oxyquinolate[7], and phenyl mercuric borate[8], and indicated different antifungal concentrations. The resistant strains require high concentrations for 100 per cent inhibition. Therefore, little higher doses i. e., more than inhibitory concentration is always recommended for commercial uses. Recently Sharma and Sharma[2] reported a new antifungal chemical viz., β-hydroxynaphthaldehyde which is effective at 0.01 per cent concentration and recommended 0.03 per cent concentration for the manufacture of fungal resistant leather.

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