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EFFECT OF CYANURATES OF DIFFERENT METALS AGAINST ASPERGILLUS NIGER BY TOXIC AGAR DIFFUSION METHOD

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Primary, secondary and tertiary cyanurates of sixteen different metals and of ammonium were tested for their fungicidal properties against *Aspergillus niger* Van Teigh. The primary cyanurate of copper and all the three cyanurates of cadmium and mercury were found effective in 0.1% concentration. The secondary and tertiary cyanurates of copper, and primary, secondary and tertiary cyanurates of nickel and lead inhibited *A. niger* at 0.5% concentration. The fungicidal action of all these cyanurates was tested by toxic agar diffusion method.

The fungicides used against common pathogenic fungi are fairly soluble in water. These fungicides, however, cannot be used against fungal attack especially in high rainfall areas where wood and other building material, in major part of the year, is exposed to high moisture conditions and the fungicide from paints etc. tends to be washed-off fairly soon after application. In these investigations 50 metallic cyanurates in their primary, secondary and tertiary forms have been evaluated against *Aspergillus niger* Van Teigh for their fungicidal properties. These compounds are scarcely soluble in water, hence they have a promise to prove effective fungicidal agents under damp conditions.

Material and Methods

For screening the primary, secondary and tertiary cyanurates of calcium, zinc, nickel, tin, cobalt, barium, magnesium, antimony, copper, chromium, lead, mercury, iron, cadmium, thorium, sodium and ammonium, toxic agar diffusion method was used. The primary, secondary and tertiary compounds of the above metals have been designated as 1a, 1b, 1c, 2a, 2b, 2c and so on respectively.

The test compounds[1] were compared with zinc oxide. The concentrations of zinc oxide used in the experiments were 0.020, 0.030, 0.040, 0.050, 0.060, 0.080 and 0.10% of the active ingredient.

A few cyanurates were tested at random for determination of a general toxic range. The concentrations of the toxic compounds used in this experiment, ranged from 0.01 to 1% with a difference of 0.01% in each successive dilution. The concentration range from 0.02% to 1% was found suitable for evaluation of fungicidal properties of all cyanurates. In subsequent experiments, method of Ashrafi *et al.*[2] was used with certain modifications. The different concentration of all test compounds were the same as used for zinc oxide and were prepared in 20 ml of Sabourad's agar, instead of 15 ml agar as reported in the original method. These toxic agar plates alongwith nontoxic Subourad's agar control plates were kept at 10°C for 1 hr instead of at room temperature as in the original method because agar did not solidify rapidly at room temperature, allowing the insoluble cyanurates to settle at the bottom of petri dish which gave erroneous results. A thick spore suspension of *Aspergillus niger* was prepared in sterile water at 30°C and 0.01 ml of this suspension was centrally inoculated in each petri dish. Direct spore inoculation as in the original method was not used as the spores tend to fall enroute to inoculation and make growth zone measurements difficult. Observations were noted till the growth in the control plate reached a maximum diameter of nine centimetres. Morphological changes such as spore colour change, restricted or wrinkled growth zone and the effect on the rate of sporulation were also observed.

Results

The compounds which did not permit the growth of *Aspergillus niger* in Sabourad's agar at 0.1% conc and above were termed 'highly toxic' the compounds which were effective in inhibiting the growth of *Aspergillus niger* at 0.5% conc and above were termed 'medium toxic' and the compounds which did not check the growth of the test fungus at 1% concentration were termed 'least toxic'.

It was also noted that at 0.08% conc of primary copper cyanurate, the growth of *Aspergillus niger* was wrinkled, raised and suppressed. At 0.06% concentration of cadmium cyanurates, the growth of *Aspergillus niger* was highly suppressed with a translucent halo round the growth after 8 days of inoculation. The cyanurates of mercury suppressed mycelial growth and sporulation at 0.06% concentration.

The primary cyanurate of copper and primary, secondary and tertiary cyanurates of cadmium and mercury were found to be 'highly toxic' (Table 1). The most effective compound was 12b which inhibited 96.7% growth of the test fungus at 0.06% concentration in comparison with the control. Comparing the fungi toxic action of

TABLE 1.—PERCENT INHIBITION OF *Aspergillous niger* BY PRIMARY CYANURATE OF COPPER, PRIMARY, SECONDARY AND TERTIARY CYANURATES OF MERCURY AND CADMINUM IN COMPARISON WITH ZINC OXIDE (ZnO).

Conc. %	Test compounds							Zinc oxide
	9a	12a	12b	12c	13a	13b	13c	
0.02	0.0	33.4	42.3	32.3	78.9	74.5	86.7	33.4
0.03	0.0	37.0	48.5	38.9	83.4	78.0	88.0	33.4
0.04	0.0	40.0	51.2	53.4	85.6	82.0	90.0	33.4
0.05	0.0	44.5	51.2	68.0	88.9	85.6	94.0	33.4
0.06	11.2	95.6	96.7	88.9	94.5	88.9	94.5	66.7
0.08	44.5	100	100	100	100	100	100	66.7
0.1	100	100	100	100	100	100	100	72.3
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 2.—PERCENT INHIBITION OF *Aspergillus niger* BY CYANURATES OF NICKEL, COPPER AND LEAD AFTER ONE WEEK OF INOCULATION.

Test compounds	Conc. %							Control	Remarks
	0.08	0.1	0.2	0.3	0.5	0.7	1.0		
3a	0.0	55.6	—	77.8	87.8	100	100	0.0	Complete suppression of sporulation.
3b	0.0	57.8	—	88.9	94.5	100	100	0.0	Growth in the form of a granular
3c	0.0	44.5	—	86.7	94.5	100	100	0.0	sticky mass
9b	0.0	33.4	88.9	100	100	100	100	0.0	Wrinkled, raised and suppressed
9c	0.0	44.5	91.2	100	100	100	100	0.0	growth
11a	0.0	0.0	—	84.5	90.1	100	100	0.0	Highly reduced growth with a trans-
11b	0.0	0.0	—	83.4	90.1	100	100	0.0	parent halo round the colony
11c	0.0	0.0	—	81.2	90.1	100	100	0.0	

copper, cadmium and mercury compounds, it is evident from Table 1 that the cadmium compounds are the most effective. The cyanurates were compared with zinc oxide, phenyl mercury acetate and phenyl mercury chloride. Only results obtained with zinc oxide have been given in Table 1 as phenyl mercury compounds inhibited growth of *Aspergillus niger* at all test concentrations.

The secondary and tertiary compounds of copper and primary, secondary and tertiary compounds of nickel and lead were found to be 'medium toxic' (Table 2). In the medium toxic range 3b was found to be the most effective and inhibited 94.5% fungal growth at 0.5% concentration in comparison with the control. The compounds of nickel were found to be more fungi toxic than those of lead.

The cyanurates of nickel at 0.5% concentration completely suppressed the sporulation of *Aspergillus niger* and the mycelial growth was observed in the form of a granular sticky mass. The secondary and tertiary cyanurates of copper at 0.5 and 0.2% concentration respectively, suppressed and tertiary cyanurates of lead at 0.5% conc highly restricted the growth of *Aspergillus niger* and the colonies showed a transparent halo round them.

Discussion

The metallic and non-metallic cyanurates, studied in these experiments for their fungicidal action have been compared with zinc oxide, phenyl mercury acetate and phenyl mercury chloride. In all the compounds tested, phenyl mercury salts inhibited *Aspergillus niger* at all test concentrations. Zinc oxide, which is at present being widely used for inhibiting fungal growth in paints[3] was less effective than cyanurates of copper, cadmium and mercury. At 0.1% concentration, zinc oxide gave 72.3% inhibition of *A. niger* while primary cyanurate of copper, primary, secondary and tertiary cyanurates of mercury and cadmium showed 100% inhibition of this fungus at this concentration.

Owen[4] has shown that copper dimethyl dithiocarbamate inhibits citrate metabolism in spores of *Neurospora sitophila*. Organic salts of mercury have been reported[5] to react with sulphhydryl dependent enzymes. Like the dithiocarbamates, the suppression of sporulation, change of colour of sporulation, change in the texture of growth of *A. niger* by cadmium, mercury, nickel and lead salts strongly points to the inactivation of enzymes in the fungus cell. Similar findings have been reported by Miller.[6]

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