Effect of Different Concentrations of Fungicides on Growth of Cellulolytic Fungus *Aspergillus niger*

Dr. Arti Saxena

Associate Professor & Head, Department of Chemistry, A.N.D.N.N.M. College, Kanpur

***Abstract* – Microorganisms, mainly fungi, are responsible for the biodegradation of various cellulosic materials, like cotton textiles, linen goods, paper and pulp products, rubberized articles, leather, rexine and plastic products, such as ropes, twines, water proof covers and many other articles used in defence services under tropical and sub-tropical conditions. Even the electronic components have not been spared from fungal damage. The damage caused by the attack of microorganisms on cellulosic materials is so enormous that the use of fungicides becomes inevitable. The present work deals with the effect of two potent fungicides- - m-Dinitro benzene and Pentachloro phenol on the growth of a highly cellulolytic fungus *Aspergillus niger*.**

***Index Terms* – m-Dinitro benzene, Pentachloro phenol, Inhibition.**

I. INTRODUCTION

Various cellulolytic fungi cause severe deterioration in indoor environments. They attack textiles, resulting in loss of fiber strength and actual material failure. Other spoilage can occur as a result of the permanent staining from pigmentation and mycelial penetration by cellulolytic fungi. The division of cellulolytic fungi into strong and weak forms is based on their ability to reduce the tensile strength of a fabric under laboratory experimental conditions. Fungi occur abundantly in soils, particularly those rich in organic matter and are very active in the decomposition of plant and animal remains. They are carried away by wind along with dust which settles on materials and brings about infection. Due to their ability to grow under adverse environmental conditions along with their high cellulolytic activity, the fungi belonging to Fungi Imperfecti group, play a major role in the deterioration of cellulose. As such, it becomes necessary to curb the activities of these microorganisms and to inhibit the activities of enzymes produced by them. As the degradation occurs at the point of contact of the fungus with the fibers of cellulose, an interruption of this contact by an inert physical barrier may prevent the fungal breakdown. Incorporation of toxic substances in the cellulosic fiber may also prevent the degradation. Suitable fungicides are used to curb the activities of the microorganisms. The performance of various fungicides and rot proofing agents have been reviewed by Weatherburn (1947) and Siu (1951).

II. EXPERIMENTAL

I.Materials & Method:

Chemicals – All the inorganic chemicals used were of analytical grade obtained from BDH Laboratories Bombay, India. Carboxymethyl cellulose was obtained from Loba Chemie Indo Austranal.

Microorganisms – The cellulolytic fungus *Aspergillus niger* was obtained from National Sugar Institute, Kanpur. The culture was grown on potato dextrose agar slants containing filter paper strips as cellulosic substrate at a temperature of 30 ± 2 °C for a period of ten days and maintained at 4 °C by subculturing every month.

Culture Conditions & Growth Media –
From ten days cultures, spore's suspension was prepared by adding sterile distilled water to the culture tubes under aseptic conditions. The suspension was filtered through sterile muslin cloth. Three milli liters of spore's suspension was transferred to a 500 ml Erlenmeyer Flask containing 100 ml of sterile medium adjusted to pH under aseptic conditions and incubated at 30 ± 2 °C on rotatory shaker (200 cycles/ min) for a period of ten days.

Preparation of stock solution of fungicides –

a) A stock solution of 1.5 X 10^{-2} M (3.9990 g/L) Pentachloro phenol (PCP) was prepared in ethanol and subsequently diluting with distilled water to get the PCP of desired percent concentration. The solution was stored in air tight glass stoppered bottles at 20 °C.
b) A stock solution of 1.5 X 10^{-2} M (2.5200 g/L) m-Dinitro benzene (MDB) was prepared in isopropanol and subsequently diluting with distilled water to get the MDB of desired concentration. The solution was stored in air tight glass stoppered bottles at 20 °C.

Techniques used to study the inhibitory effect of fungicides –

Different concentrations of the two fungicides were added to the broth and potato dextrose agar media to observe the qualitative and quantitative growth of the fungal cultures.

II. Result and Discussion:

Different concentrations of the two fungicides MDB & PCP were added to the culture filtrates. Molar concentrations ranging from 0.5 X 10^{-6} M to 9.0 X 10^{-6} M of the two fungicides were added to the sterile medium, prior to inoculation. The flasks were then incubated on rotatory shaker at 30 ± 2 °C. Control flasks without any fungicide, were also taken simultaneously. The growth of the fungus was measured in terms of mycelial dry weight in mgs/100ml of the medium and the results obtained are recorded in the Table.

From the Table it is clear that 6.0 X 10^{-6} M solution of m-dinitrobenzene completely inhibits the growth of *Aspergillus niger* while higher concentration (7.5 X 10^{-6} M) of pentachloro phenol is required to completely check its growth.

Thus m-dinitrobenzene is a stronger fungicide than pentachloro phenol, as much higher concentration of later than that of the former is required to check the growth. Kowalik & Grodek (2002) studied the effect of various fungicides, such as, Topsin, Bravo & Sportak on the growth of some fungi including *Aspergillus niger* isolated from infected in vitro cultures of fruit bearing plants. Pentachloro phenol was used as a preservative for paint by Hoffman et al. (1966). Dholakia and Chhatpar (1980) reported PCP, 8 (OH) quinoline and dimethyl formamide to be inhibitive towards the growth of *Aspergillus* sp. grown on water-based poster colors. They found that in addition to these fungicides phenyl mercuric acetate could also inhibit the growth. In 1978, Patwa et al. found that 100 µg/ ml of *Cryptococcus neoformans* and *Saccharomyces cerevisiae* but in order to inhibit the growth of *Aspergillus niger*, 200 µg /ml of these fungicides were required.

III. CONCLUSION

The growth and sporulation of the cellulolytic fungus *Aspergillus niger* was found to be inhibited by using two fungicides, i.e., m-dinitrobenzene and pentachloro phenol. 6.0 X 10^{-6} M concentration of MDB could completely inhibit the growth of *Aspergillus niger* while PCP of 7.5 X 10^{-6} M concentration was needed to completely check its growth.

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Table XI

Effect of different concentrations of fungicides on growth of Aspergillus niger

Concentration of fungicide (M)	Mycelial dry weight (mgs/100 ml)	
	MDB	PCP
0.0	380	380
0.5×10^{-6}	240	299
1.5×10^{-6}	182	203
3.0×10^{-6}	55	70
4.5×10^{-6}	12	35
6.0×10^{-6}	-	15
7.5×10^{-6}	-	-
9.0×10^{-6}	-	-

\- indicates no growth

MDB = m-dinitrobenzene.

PCP = pentachlorophenol.