Efficacy of Homoeopathic Drugs for the Control of Fungal Growth on Cellulosic Materials

K.L. Garg and S. Dhawan***

Abstract

Cultural property made of cellulosic materials is more subjected to bio-deterioration in tropical and sub-tropical countries because of humid and warm climate. The most severe deterioration of paper and textile objects is brought about by fungi having high cellulolytic activity. Use of chemical biocides for controlling the growth of fungi may have adverse effect on cellulosic objects and further they have potential explosion hazard to the users. Homoeopathic drugs have long been used for the treatment of human diseases of fungal origin. Therefore, to prevent fungal growth on paper, five homoeopathic drugs viz., Tellurium, Psorinum, Arsenicum, Rumex and Graphitis were tested for their antifungal efficacy against cellulolytic fungi isolated from deteriorated paper and textile materials. Out of 16 fungi so isolated, three, namely, Aspergillus niger, Chaetomium globosum and Emericella nidulans were found to be most frequent and had highest cellulose activity. During in vitro testing, two homoeopathic drugs, Arsenicum and Rumex showed maximum inhibition of fungal growth. These two homoeopathic drugs were also found effective against all the three fungal isolates, under in situ conditions, even after one year of incubation. Both the homoeopathic drugs were also tested on book pages damaged by fungi and encouraging results were obtained.

Key-words *:* Homoeopathy, Anti-fungal activity.

1. Introduction

Large collections of paper and textile objects are available in museums, archieves and libraries of India and Southeast Asia. Paper and textile are complex organic materials composed mainly of cellulose. Cellulose is a linear polysaccharide (made of carbon, hydrogen, and oxygen) consisting of D-glucose units linked together in long chains by (1-4-ß) glucosidic linkages. The problem of biodeterioration of cellulosic material is more prominent in tropical and subtropical regions where temperature and humidity conditions favour the growth of fungi. The most severe deterioration in indoor environment is primarily caused by fungi having high cellulolytic activity. These fungi have remarkable cellulose dissolving capacity, which in turn weakens the strength of cellulosic objects and may cause staining or discoloration (Mahomed, 1971; Kowalik, 1980; Vigo, 1980; Gallo, 1985, 1994; Nyuksha, 1994). Conservation treatment on the paper and textile objects by different museums, libraries throughout the world have proven that no chemical treatment is safe enough in controlling fungi on cellulosic materials (Caneva et al., 1991; Montegut, et al., 1991). Homoeopathic drugs have been used in plant disease control due to their being antimicrobial, non-toxic and non-hazardous to the environment. (Khanna and Chandra, 1977, 1980; Goswami and Das 1980). Their antifungal activity for the protection of books and paper materials have been investigated only recently (Garg, 1995).

Therefore, a study was conducted to observe the antifungal activity of homoeopathic drugs against fungi isolated from deteriorated paper and textile objects under in vitro and in situ conditions.

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2. Materials and Methods

2.1. Isolation and Purification of Cellulolytic Fungi.

Cellulolytic fungi were isolated from infected old book and cellulosic textile by standard microbiological and non-destructive techniques (Dhawan and Agrawal, 1986). Isolated fungi were purified and identified with the help of a microscope.

2.2 Screening of Fungal Isolates for Cellulose Activity.

Cellulolytic activity of each fungal isolate was determined as per method of Morral, et.al.(1972). Modified Richard's medium containing 1% cellulose powder as a sole carbon source was used. pH of the medium was adjusted to 6.5. 25 ml of this medium was dispersed into 100ml flasks which were autoclaved at 15lbs/sq. inch pressure for 20 minutes. Flasks were inoculated with the fresh inoculum of the tested fungal forms and were incubated at 28±1oC. Ten days old cultures were passed through seitz filter and 2ml of the culture filtrate (enzyme extract) was added to 10ml of buffer substrate mixture i.e. 1% sodium carboxymethyl cellulose (Na -CMC) prepared in 0.1M citrate buffer (pH 4.5) and incubated for one hr at 30°C. Cellulase enzyme activity was assayed viscometerically using Oswald viscometer. Boiled enzyme was used for the control experiment. Cellulose activity was expressed in terms of percent reduction in viscosity of reaction mixture. One unit of enzyme activity was defined as the amount of enzyme causing 1% loss of viscosity of reaction mixture after incubation for one hour.

2.3 Testing of Homoeopathic Drugs against Cellulolytic Fungal Isolates under in vitro and in situ conditions.

Various potencies of five homoeopathic drugs viz., Tellurium, Psorinum, Arsenicum, Rumex and Graphitis were tested for their antifungal activity against most frequently occurring fungi isolated from badly deteriorated book and cellulosic textile under in vitro conditions by using Poisoned Food Technique on cellulose agar medium. The technique used was similar to that reported earlier by Garg, 1995.

For in situ testing of their effective potencies, selected homoeopathic drugs were tested for their antifungal property on three types of cellulosic textiles viz., canvas, muslin and cotton cloth and three types of paper viz., Japanese, Nepalese and pure cellulose. One inch square of paper and textile pieces were treated with effective potencies of homoeopathic drugs. These treated paper and textile pieces were inoculated by a mixture of fungal spores (A. niger, C. globosum and E. nidulans) and kept in a humidity chamber at 28±1oC. Arsenicum 30, 200 and Rumex 200, 1M homoeopathic drugs were also applied on an old discarded book affected by fungi and placed in a humidity chamber at 28±1oC. In control set, no homoeopathic drug was applied. Observations were made after 30 days intervals.

Table-1

Frequency of occurrence of fungi on deteriorated cellulosic materials

Name of Fungi	Frequency of occurrence	
	Textile	Paper
Alternaria alternate (Fr.) Keissl.	-	++
Aspergillus niger Van Tieghem	++++	++++
Cephalosporium sp.	-	+
Chaetomium globosum Kunze ex Steud.	++++	++++
Cladosporium herbarum (Pers.) Link ex S.F. Gray	++	++
Curvularia lunata (wakker) Boedijn	-	+++

Drechslera hawaiiensis (Bugnicourt) Subram & Jain ex M.B. Ellis, Subram. and Jain	+++	-
Emericella nidulans (Eidam) Vuillemin	++++	++++
Fusarium solani (mart.) Sacc.	++	-
Memnoniella echinata (Riv.) Galloway	+	-
Paecilomyces variotii Bain.	+	+
Penicillium citrinum Thom	+	-
Scopulariopsis brevicaulis (Sacc.) Bain.	++	-
Stachybotrys atra Corda	-	++
Starchiomyces sp.	-	+
Trichoderma viride Pers. Ex Gray	-	++

Note:
++++=Very Good,
+++=Good,
++=Fair,
+=Poor,
-=Not isolated

Table-2

Cellulolytic activity of fungi isolated from cellulosic materials

Name of Fungi	Cellulase activity (Unit/ml) Textile	Paper
Alternaria alternate	-	53.0
Aspergillus niger	91.0	93.5
Cephalosporium sp.	-	19.5
Chaetomium globosum	92.8	91.5
Cladosporium herbarum	37.0	42.0
Curvularia lunata	-	49.5
Drechslera hawaiiensis	49.0	-
Emericella nidulans	87.0	89.6
Fusarium solani	47.2	-
Memnoniella echinata	27.5	-
Paecilomyces variotii	21.6	34.0
Penicillium citrinum	23.0	-
Scopulariopsis brevicaulis	43.5	-
Stachybotrys atra	-	51.0
Starchiomyces sp.	-	21.6
Trichoderma viride	-	41.5
Control	Nil	Nil

3. Results and Discussion

The degradation of cellulose is brought about by heterotrophic microorganisms, possessing a system of extracellular and intracellular enzymes known as cellulases. The most active agents of paper and textile deterioration are microfungi. Large number of fungal spores are present in the air of museums, libraries and archives. Acidic pH and humid conditions with favourable temperature favour the growth of cellulolytic fungi on paper and textile objects. The fungi growing on cellulosic materials viz., paper and textile cause several kinds of alterations such as discoloration, staining and loss of strength. Among cellulolytic strains of fungi, many species of Alternaria, Aspergillus, Fusarium, Memnoniella, Penicillium, Scopulariopsis, Stachybotrys, Stemphylium, Trichoderma and Chaetomium have been frequently isolated from paper and cellulosic textile materials (Kwalik, 1980; Vigo, 1980; Gallo, 1985, 1994; Nyuksha, 1994; Garg, 1995).

The first experiment was done for the isolation and identification of fungi from badly deteriorated book and textile objects (Figs. 1,2). Table 1 shows the frequency of occurrence of cellulolytic fungi on deteriorated paper and textile materials. Aspergillus niger, Chaetomium globosum and Emericella nidulans were found to be the most frequently occurring fungi both on textile as well as paper material. Cellulolytic activity of various fungal isolates was determined and compared, it was found that A. niger, C. globosum and E. nidulans have highest cellulose activity (Table-2). This observation supported the results of frequency of their occurrence. Since these fungi had higher cellulolytic activity, they were found more frequently on paper and textile materials. This shows that primary attack was caused by these fungi. Other fungi showing lower frequency of occurrence and lesser cellulolytic activity might have come as secondary infection to consume glucose and other simpler metabolites produced by cellulolytic fungi.

In vitro testing of various potencies of homoeopathic drugs against A. niger, C. globosum and E.nidulans was done. It was observed that out of five homoeopathic drugs tested, Arsenicum 200, 30 and Rumex 1M, 200 showed maximum effectiveness (Tables-3,4 and Figs. 3,4). These two homoeopathic drugs viz., Arsenicum 200, 30 and Rumex 1M, 200 were found effective under in situ conditions also when applied on paper and textile, each three type, and inoculated with spores of cellulolytic fungi (Tables-5,6 and Figs.5 to 9). These two homoeopathic drugs were also used for the control of fungal growth on pages of a deteriorated book and found effective (Figs. 10,11).

Table - 3

In vitro testing of antifungal activity of homoeopathic drugs against Some fungal forms isolated from book

Homoeopathic drug		Percentage inhibition of fungal growth		
Name	Potency	Aspergillus Niger	Chaetomium globosum	Emericella nidulans
Tellurium	1M	10	25	20
	200	10	25	Nil
	30	Nil	10	Nil
Psorinum	1M	Nil	Nil	Nil
	200	Nil	Nil	Nil
	30	Nil	Nil	Nil
Arsenicum	10M	Nil	20	Nil
	1M	90	80	90
	200	100	100	100
	30	100	100	100

Rumex	1M	100	100	100
	200	100	100	90
	30	40	50	40
Graphitis	1M	40	50	25
	200	Nil	20	15
	30	Nil	10	10
Control (without any homoeopathic drug)	-	Nil	Nil	Nil

Table - 4

In vitro testing of antifungal activity of homoeopathic drugs against some fungal forms isolated from cellulosic textile

Homoeopathic drug		Percentage inhibition of fungal growth		
Name	Potency	Aspergillus Niger	Chaetomium globosum	Emericella nidulans
Tellurium	1M	15	20	20
	200	Nil	10	Nil
	30	Nil	Nil	Nil
Psorinum	1M	Nil	Nil	Nil
	200	Nil	Nil	Nil
	30	Nil	Nil	Nil
Arsenicum	10M	10	20	Nil
	1M	90	80	80
	200	90	100	100
	30	100	100	100
Rumex	1M	100	100	100
	200	100	100	100
	30	50	60	40
Graphitis	1M	25	40	15
	200	10	20	15
	30	Nil	10	10
Control (without any homoeopathic drug)	-	Nil	Nil	Nil

Table - 5

In situ testing of efficacy of Arsenicum and Rumex on Various type of papers

Homoeopathic drug		Observations of fungal growth after 3 months interval											
Name	Potency	Nepalese				Japanese				Pure cellulose			
		3	6	9	12	3	6	9	12	3	6	9	12
Arsenicum	10M	-	+	++	+++	-	+	++	+++	-	+	++	+++
	1M	-	-	+	++	-	-	+		+	-	-	+ +
	200	-	-	-	-	-	-	-		-	-	-	- -
	30	-	-	-	-	-	-	-		-	-	-	- -
Rumex	1M	-	-	-	-	-	-	-		-	-	-	- -
	200	-	-	-	-	-	-	-		-	-	-	- -
	30	-	+	++	+++	-	+	++	++	-	+	++	++
Control (Untreated)	-	+	+++	++++	++++	-	++	+++	++++	-	++	+++	++++

Note:

-	=	No growth	+	=	Poor growth
++	=	Fair growth	+++	=	Good growth
++++	=	Very good growth			

Table - 6

In situ testing of efficacy of Arsenicum and Rumex on various type of textiles

Homoeopathic drug		Observations of fungal growth after 3 months interval Type of cellulosic textile pieces											
Name	Potency	Canvas				Muslin				Pure cotton			
		3	6	9	12	3	6	9	12	3	6	9	12
Arsenicum	10M	-	+	++	+++	-	+	+	+++	-	+	++	+++
	1M	-	-	+	++	-	-	+		+	-	+	+ ++
	200	-	-	-	-	-	-	-		-	-	-	- -
	30	-	-	-	-	-	-	-		-	-	-	- -
Rumex	1M	-	-	-	-	-	-	-		-	-	-	- -
	200	-	-	-	-	-	-	-		-	-	-	- -
	30	-	+	++	+++	-	-	+		+	-	+	+ ++
Control (Untreated)	-	++	+++	++++	++++	+	++	+++	++++	+	++	+++	++++

Note:

-	=	No growth	+	=	Poor growth
++	=	Fair growth	+++	=	Good growth
++++	=	Very good growth			

The in vitro and in situ testing of antifungal effectiveness of Sulphur Iodatum 1M and Petroleum 30 against cellulolytic fungi namely Aspergillus niger was first reported by Gard (1995). Sulphur Iodatum 1M has been reported to be effective against two plant pathogenic fungi (Singh and Gupta, 1981). Singh and Gupta (1981) also reported that various potencies of Bacillinum, Petroleum and Mezerium were effective against Alternaria tenuis and Curvularia lunata fungi. The present study adds to the list of homoeopathic drugs which could be used safely in controlling the fungal deterioration of cultural objects like paper and textile materials.

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References

- Caneva, G., Nugari, M.P. and Salvadori, O. (1991). Biology in the Conservation of Works of Art . Rome, Italy, ICCROM.
- Dhawan, Shashi and Agrawal, O.P. (1986). Fungal Flora of Miniature Paper Paintings and Lithographs. International Biodeterioration, 22(2) : 95-99.
- Gallo, F. (1985). Biological Factors in Deterioration of Paper. Rome, Italay, ICCROM.
- Gallo, F.(1994). The Biodeterioration of Library Materials, In : Recent Advances in Biodeterioration and Biodegradation, Vol.1, (Eds.) K.L. Garg, N. Garg and K.G. Mukerji, Calcutta, India, Naya Prokash, PP. 89-143.
- Garg, K.L.(1995). Use of Homoeopathic Drugs as Antifungal Agent for the Protection of Books and Paper Materials, In : Biodeterioration of Cultural Property 3, Thailand, July 4-7, 1995, Bangkok. (Eds.) C. Aranyanak and C. Singhasiri, Bangkok, Thailand, Conservation Science Division, Na Phra That Road, pp.103-115.
- Goswami, N. and Das, D.(1980). Possibilities of Homoeopathic Treatment in Plants and Animal Diseases, Hahnemannian Gleaning, XLVII : 332-341.
- Khanna, K.K. and Chandra, S. (1977). Control of Guava Fruit Rot Caused by Gloeosporium psidii with Homoeopathic Drugs, Plant Dis. Reporter, 61 : 362-366.
- Khanna, K.K. and Chandra, S.(1980). A Homoeopathic Drug (Lycopodium) Controls Mango Fruit Rot Caused by Pestalotia mangiferae Hann. Hahnemannian Gleanings, XLVII : 441-448.
- Kowalik, R. (1980). Microbiodecomposition of Basic Organic Library Materials. Microbiodeterioration of Library Materials, Part 2, Restaurator, 4 : 135-219.
- Mahomed, R.S.(1971). Antibacterial and Antifungal Finishes. In : Chemical After Treatment of Textiles, (Eds.) H. Mark, N.S. Wooding and S.M. Atlas. New York, London, Wiley Interscience, pp. 507-552.
- Montegut, D., Indictor, N. and Koestler, R.J. (1991). Fungal Deterioration of Cellulosic Textiles : a Review, Int. Biodet. 28 : 209-226.
- Morral, R.A.A., Duczke, L.J. and Sheared, J.W.(1972). Variation and Correlations within and between Morphological, Pathogenecity and Pectolytic Enzyme Activity in Sclerotinia from Sackstechewan, Can. J. Bot., 50 : 767-786.
- Nyuksha, Ju.P.(1994). The Biodeterioration of Paper and Books, In : Recent Advances in Biodeterioration and Biodegradation, Vol. 1. (Eds.) K.L. Garg, N. Garg and K.G. Mukerji, Calcutta, India, Naya Prokash, pp. 1-88.
- Singh, B.S. and Gupta, G.(1981). Effect of Homoeopathic Drugs on the Growth of Alternaria tenuis Auct. And Curvularia lunata (Wakker) Boedijn, the Common Leaf Spot Pathogens of Ornamental and Cultivated Plants, Hahnemannian Gleanings, XLVIII : 411-413.
- Vigo, T.L.(1980). Protection of Textiles from Biodeterioration, Conservazione e Restauro dei Tessili Convegno Internazionale, Como, 18-26.

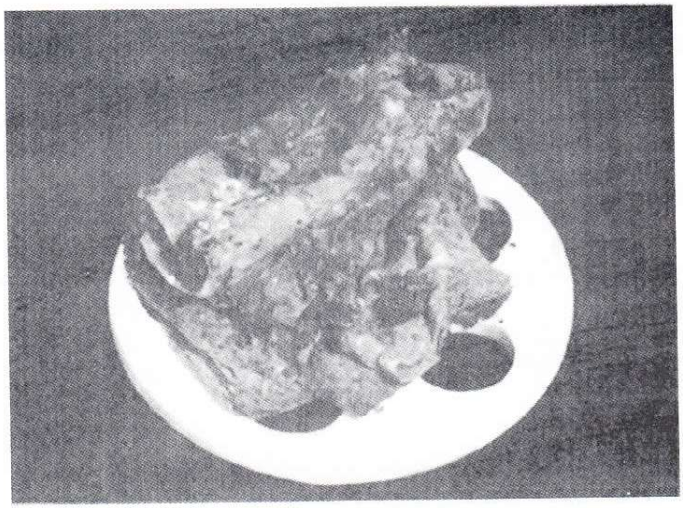

Fig. 1. Cellulosic textile damaged by fungi.

Fig. 2. Book affected by fungal growth.

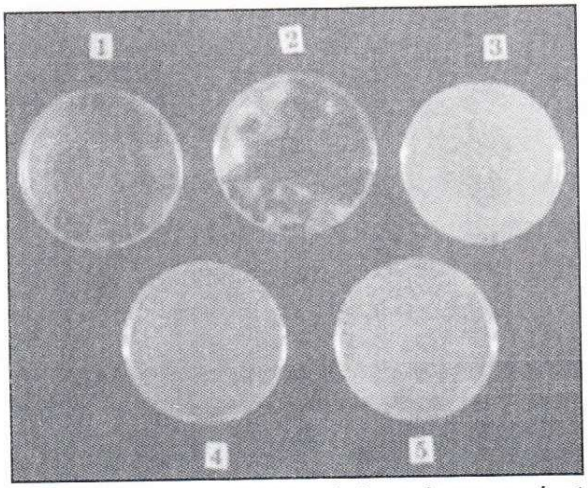

Fig. 3. *In vitro* efficacy of Arsenicum against *Aspergillus niger* (1) Control, (2) 10M, (3) 1M, (4) 200 and (5) 30 potencies.

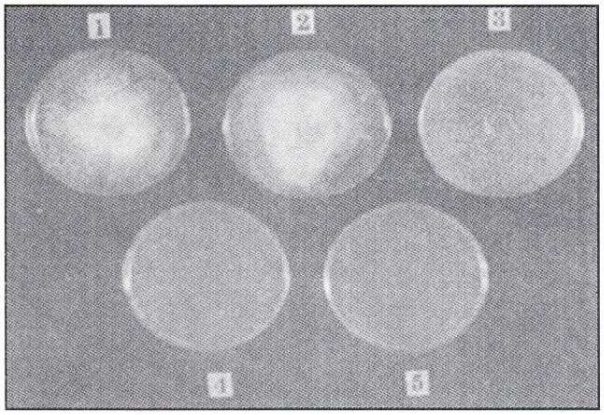

Fig. 4. *In vitro* efficacy of Arsenicum against *Emericella nidulans* (1) Control, (2) 10M, (3) 1M, (4) 200 and (5) 30 potencies.

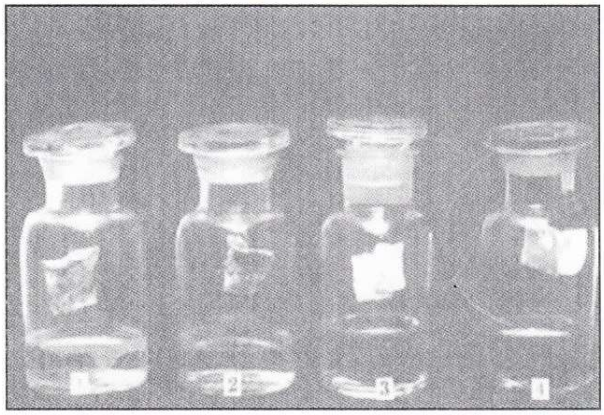

Fig. 5. Experimental set up of *in situ* studies of efficacy of homoeopathic drugs against fungi.

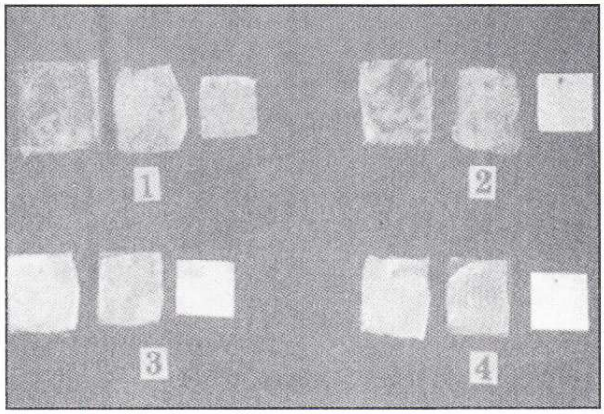

Fig. 6. *In situ* efficacy of Arsenicum on cellulosic textiles against fungi (1) Control, (2) 1M, (3) 200 and (4) 30 potencies.

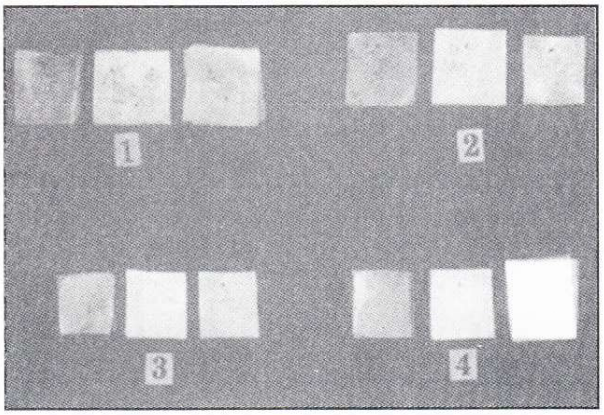

Fig. 7. *In situ* efficacy of Arsenicum on papers against fungi (1) control, (2) 1M, (3) 200 and (4) 30 potencies.

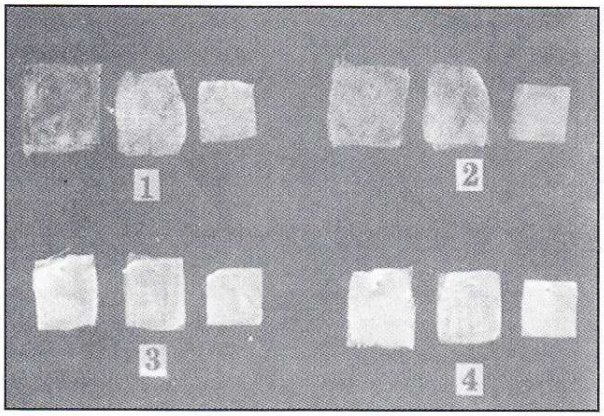

Fig. 8. *In situ* efficacy of Rumex on textiles against fungi (1) control, (2) 30, (3) 200 and (4) 1M potencies.

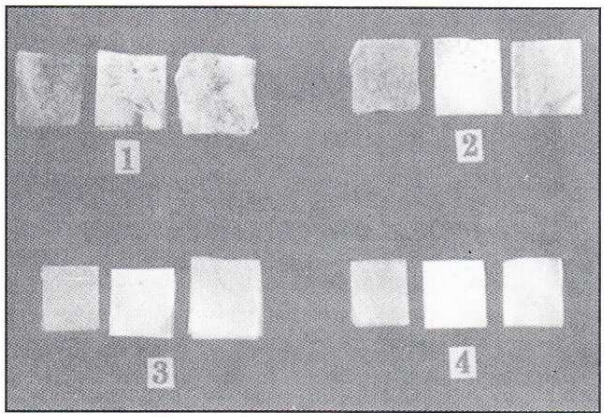

Fig. 9. *In situ* efficacy of Rumex on papers against fungi (1) control, (2) 30, (3) 200 and (4) 1M potencies.

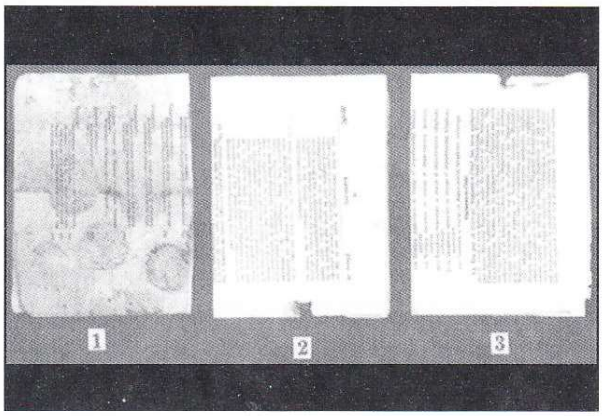

Fig. 10. Efficacy of Arsenicum on book pages affected by fungi (1) control, (2) 200 and (3) 30 potencies.

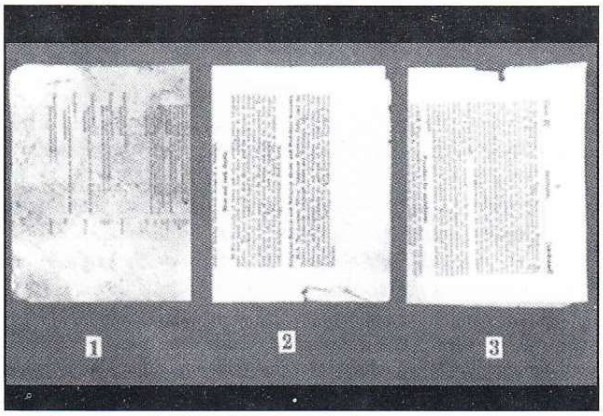

Fig. 11. Efficacy of Rumex on fungal affected book pages (1) control, (2) 200 and (3) 1M potencies.