Isolation and Identification of Fungal Species Affecting Leather Garments and Evaluation of Effective Fungicides

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Abstract

Leather and leather products can be damaged by bacteria and fungi, which thrive on tanned leathers containing carbohydrates, fats and proteins. A number of fungicides are now available to inhibit the growth of these moulds on organic substrates. Ecological and safety concerns are being raised about many of the conventional fungicides. The present study focuses on use of combination of two safe fungicides. It is recommended from the study that a combination of these fungicides demonstrate synergistic effect in controlling the fungal species that attack leather and leather garments.

Introduction

Biodeterioration is an important factor impairing aesthetic, functional and other properties of leather and the products made from them, Orlita (2004). Finished garment leathers provide an excellent substratum for mould growth. The presence of lubricating agents and other organic materials may tend to promote microbial growth on leather. Moulds may also appear during drying in favourable humidity or if the drying process takes too long. However there are new biocidal alternatives which are more sensitive to the conditions of application (Rother,1995; Lindner, 1998).Para nitro phenol (PNP) and pentachlorophenol (PCP) which have been used for decades are considered unsatisfactory due to their toxicity (Hauber and Germann, 1997; Didato and Yanek, 1999). For any biocide to be useful in the leather industry, it should fulfil the following requirements.

High anti microbial activity
Broad antimicrobial spectrum
Compatibility with other leather auxiliaries
Stability on leather
Non discolouring
Environmentally acceptable
Low toxicity to humans
Cost effectiveness

Extensive studies have been done on mycology of the leather especially mould growth at various stages of leather. However reports on growth of mould on stored leather garments is less.

The fungi occurring on the leather are generally grouped as moulds. Moulds grow best under warm damp conditions. The optimum temperature varies with the species but is commonly between 25^{o}C and 35^{o}C. Moulds rarely grow above 50^{o}C. They also grow in high relative humidity. Thus bio deterioration in the leather results from the activity of macro and micro organisms on raw hides during leather manufacture and also during storage of finished leathers and products. (Orlita, 1968a, 1993; Zyska, 1997,2000). Also fungal growth on finished leather can adversely affect strength properties. Loss of strength has been attributed to utilization of oils and other organic material by fungi as a part of their metabolic processes. This necessitates a safe guard of leather apparel against fungal attack.

A number of methods have been suggested for inhibiting the growth of fungi. They are changing environmental conditions or adding certain organic or inorganic substances that inhibit the growth of fungi. Changing the favourable condition of mould may not be viable in leather processing. The only practical approach is the use of certain fungicides like P-nitro phenol, beta-napthol, sodium tri chlorophenate, tricholoro phenol, salicyl anilide, pentachlorophenol, p-chlorometal xylenol and mercuric chloride, Musgrave (1948). However these compounds are reported to be effective only at higher concentrations and cause health hazards. In the present study, OPP(ortho phenyl phenol) and TCMTB (2- thiocyanomethylthio benzothiazole) have been used as they do not alter the eco system under concentrations employed . Introduction of TCMTB is found to be efficient in protecting finished leather Lakshmi et al, (2001), but under storage conditions, the development of spores was not controlled by TCMTB, Adminis et al, (2001).

In the present study, an attempt has been made to identify the fungal species showing growth on finished leather garments upon storage and evaluation of fungicides in various combinations (OPP and TCMTB) on isolated fungal species and evaluation of physical characteristics of garments infected with the isolated species.

Materials & Methods

Isolation and identification of fungal species

Sheep leather garment samples with 0.9 – 1mm thickness were chosen for the present study. The area where the fungal growth was exhibited was cropped and stored in sterilized polythene bags for experiments.

Enumeration of the fungal spores was carried out with Sabouraud Dextrose Agar with 5% NaCl (32.5 g Sabouraud Dextrose Agar; 25 g Sodium chloride; 500 ml distilled water) and Potato Malt agar (HiMedia). The cropped leather piece was cut into 1 x 1 cm^2 pieces using sterile scissors under sterile environment and was soaked in presterilized water (5 pieces in 10 ml of water) for 30 minutes under stirring condition at room temperature enabling to get all the spores adhered on the surface of the leather piece. The supernatant containing the free spores were transferred carefully in to the pre sterilized test tubes and was used as inoculum. About 10 – 20 µl of diluted

spore suspension (0.6 OD at 600 nm) was inoculated in the solidified agar media. The plates were incubated at 37°C for 48 hours. Macroscopical and microscopical evaluation (lacto phenol cotton blue staining) of the fungal species exhibiting growth was assessed. The mould species were screened and sub cultured at periodical intervals and stored at 4°C.

Evaluation of fungicides

Industrial grade Ortho-phenylphenol (OPP – Phenolic) and 2-(thiocyanomethylthio benzothiazole) (TCMTB - Heterocyclic) were used to study their efficacy on the fungal species isolated from infected leather garments. The method used for this study is based on ISO Standard method, ISO/DIS 5433 Annexure A 1994. Piece of leather garment (1x1 cm^2) samples free from fungal infection was washed thrice with sterile water and dried at 70°C. After 36 hours, the sample free from moisture was placed in a glass jar and was completely sealed. About 500 µl spore solution of six individual fungal species was sprinkled separately to each leather garment piece before sealing. Mould growth was observed for 145-180 days. Duplication of the experiments was done to confirm the results obtained.

Evaluation of deterioration of physical properties of leather

After 180-200 days of exposure, the samples exhibiting spores of isolated species has been taken out carefully and the spores were disinfected with ethanol (95%) in the air flow controlled UV chamber. The resultant garment samples free from spores were further subjected to Scanning Electron Micrograph analysis to assess the invasion and intrusion of fungal spores in the garment sample. Tensile strength and elongation at break measurements were made on the infected garment sample by conventional method using series IX automated materials testing system, Instron at a humidity of 65% and temperature of 20 ^{0}C.

Results and Discussion

Extensive studies have been done on the mycology of leather industry especially mould growth on pickled skin, vegetable and chrome tanned leather. Only scanty report is available on growth of mould on finished and stored leather garments. Though the finished products have been exposed to number of fungicides during leather processing, the growth of fungi on these products indicates that these species has developed resistance towards particular fungicides.

Birbir and Ilgar (1984), Bailey and Birbir (1996) and Russell etal (1998), have been especially concerned with halophilic microorganisms and the problem of the colouration of cured hides, but the role of halophilic and non halophilic bacteria producing or not producing coloured spots on a salted hide is not clear, because the individual types can manifest themselves successively so that their individual hydrolytic effects are hidden.

It is interesting to note that this report is the first report revealing the presence of *Chaetomium* species in finished leather products. All the available literature on mould growth specifically speaks about *Pencillium* and *Aspergillus* species. The photographs enclosed as Figure 1 gives the morphological nature of the isolated *Chaetomium species* from infected leather garments.

Moreover, the source of *Chaetomium* was reported by Frey et al (1979) and Rippon (1978) as from cow dung and the presence of this species in leather garments upon storage suggested that this species might have developed resistance towards various conventional fungicides applied during the processing of leather and also upon the storage. Lollar (1944a) suggested that incorporation of preservatives to the leather while in processing might reduce the growth of moulds. But, the occurrence of *Chaetomium* species in the garment test sample inferred that it might have come from the slaughterhouse while flaying of skin/hide and remained during from processing and again reoccurred upon storage.

The fungal species screened from infected leather garments include six mould species namely *Chaetomium, Aspergillus niger, Aspergillus terreus, Absidia and Scedosporium apiospermum* (Figure 1). Periodical assessment of infected leather garments for 12 months revealed that these six species were commonly found in the leather garments upon storage.

The in *vitro* studies on application of the fungicides (OPP and TCMTB) of varied nature and their combinations at different concentrations on six isolated fungal spores namely *Chaetomium, Aspergillus niger, Aspergillus terreus, Absidia and Scedosporium apiospermum s*howed no immediate germination of sprinkled spores in all the experimental jars. This might be due to the effectiveness of the remnants of applied fungicides during the storage of garments. The remnants of controlling agents in the leather garments prevent the growth of sprinkled spores up to 10 days but, when the incubation period increased, the fungal spores acclimatise with the prevailing environment and starts growing. This suggests that the leftover of the controlling agents could not effectively kill the applied spores. This is the reason why all the six control jars exhibited growth after 15 days irrespective of all the species studied.

In the presence of fungicides, all the isolated mould species exhibited varied sensitivity towards each fungicide and their mixture. About 7 ml of OPP and TCMTB (0.01 - 0.1%) (w/v) in aqueous solution combination chosen based on preliminary investigation (Table 1). No immediate germination of fungal spores up to 45 days was observed in all the experimental jars except control jar. But the difference in sensitivity arises at the end of 45^{th} day. The growth of mould viz., *Chaetomium* sp. isolated from the leather garment upon storage, exhibited only 45% sensitivity towards OPP. But with TCMTB the sensitivity increased to 70%. Sensitivity of the isolated species towards mixtures of fungicides was highly encouraging. Mould growth was completely prohibited by the combination of OPP and TCMTB. This suggested that due to the difference in sensitivity of the moulds towards the individual fungicides, the cumulative effect of the mixtures protected the leather garment
from mould growth

Similarly, the mould *Scedosporium apiospermum* showed growth even in the presence of fungicide and their mixtures. The sensitivity of this species towards chosen fungicide and the mixtures was only about 30-40%. Hence, it is necessary to identify the controlling agent, which could completely inhibit the growth of *Scedosporium apiospermum*.

Considering other isolated mould species, growth of *Absidia* was totally absent with combination of OPP and TCMTB. Species belonging to *Aspergillus* family showed sensitivity

both towards the individual fungicides as well as towards the mixtures. Growth of *Aspergillus terreus* was completely inhibited by OPP and TCMTB.

The quantity required for a particular fungicide to inhibit fungal species depends on a number of factors including the storage time and conditions, the thickness of the skins and the presence of nutrients such as organic materials, lubricants. The reduced action of fungicide TCMTB on selective fungal spores might be due to the reaction of TCMTB with sulphide and bisulphite present in the inner portion of the leather and getting converted to less active MTB. Scanning electron micrograph of the infected leather garments emphasised the resistant fungal species has the high opportunity to intrude into the interlinking collagen fibres.

It has also been found that any fungicide decreases in effectiveness against any particular species of mould as conditions approach the optimum for its growth. Mitra and Das (1954), Musgrave and Mitton (1950) and Robertson (1946) confirmed that the water solubles in the leather are probably the chief source of nutrient for moulds in the finished leather products and reported that the growth of mould species in between the fibre bundles in finished leather. To avoid or combat the intrusion of mould species into the collagen fibre bundles high concentration of fungicides has to be given. But, the higher concentration may affect the metal part of the garments if any and in accordance with the pollution control board regulations it is necessary to use less concentration of fungicides. At this juncture, single fungicide may not be effective; only the mixed combinations may give the satisfactory results. Lindner (1998), recommended that if the single fungicide is not effective, the combinations or the change in formulations could help to protect the materials from mould growth. The present study also proves that the cumulative effect of the mixtures of fungicides effectively control the mould growth in finished leather garment.

Scanning electron micrograph of the samples evidently proves the intrusion of fungal spores inside the collagen bundles. Due to this intrusion, the physical and surface properties of the garment sample were very much affected. It has been observed from the Table 2, which summarizes the results on the tensile strength and elongation at break for both fungicide treated and infected garment samples. The results clearly exhibited how the garment sample loses its tensile strength to the lowest value of 124.8 Kg cm^2 compared to the original value of 287.2 Kg cm^2. Similarly elongation at break was estimated as only 28% compared to 34.5 % observed for control.

Conclusion

A number of fungicides are currently used in leather industry during various unit operations. But, continuous use of these chemicals though effectively destroys the growth of mould, the health and the environmental concerns restrict their usage at required concentration levels. Moreover various new mould species exhibited growth in the finished leather garments treated with conventional fungicides. Hence, it is essential to have a fungicide effective even at lower concentration and without any adverse effect to the ecosystem. The studies, carried out indicated that the individual use of OPP and TCMTB is effective for 45 - 90 days. However a mixture of OPP and TCMTB prohibit the mould growth for a period of 180 days. Results on effect of these

mould species on deterioration of properties of finished leather garments revealed that to meet the international standards and to realise the export value of the materials, it is necessary to safeguard the leather garments free from mould attack using suitable fungicide formulation as the one developed at present.

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Table 1: Effect of Fungicides and their mixtures

Time of appearance of visibility (days)				
Organism	**Control**	**OPP**	**TCMTB**	**OPP+TCMTB**
Chaetomium	15	60	90	180
Absidia	45	145	145	-
Aspergillus flavus	15	45	90	-
Aspergillus Terreus	15	145	145	-
Pencillium	15	-	145	-
Scedosporium Apiospermum	15	45	145	-

Control – Without fungicide and their mixtures

Table 2: Organoleptic Properties of leathers

S.No	**Tensile Strength ($Kg\ cm^2$)**		**Elongation at Break (%)**	
	OPP+TCMTB	Without fungicides	OPP+TCMTB	Without fungicides
1.	287.2±0.5	124.8±0.5	34.5±1	28.2±1
2	386.3±0.5	136.5±0.5	35.7±1	24.5±1
3.	398.9±0.5	122.4±0.5	57.7±1	22.6±1

Figure I

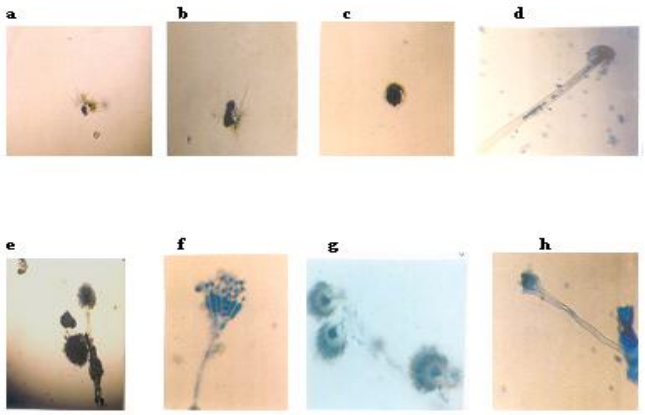

Figure 1: Fungal species isolated from infected leather garments.

a – c - *Chaetomium sp.*

d - *Absidia sp.*

e - *Aspergillus niger*

f - *Penicillium sp.*

g - *Aspergillus terreus*

h - *Scedosporium apiospermum*

Figure II

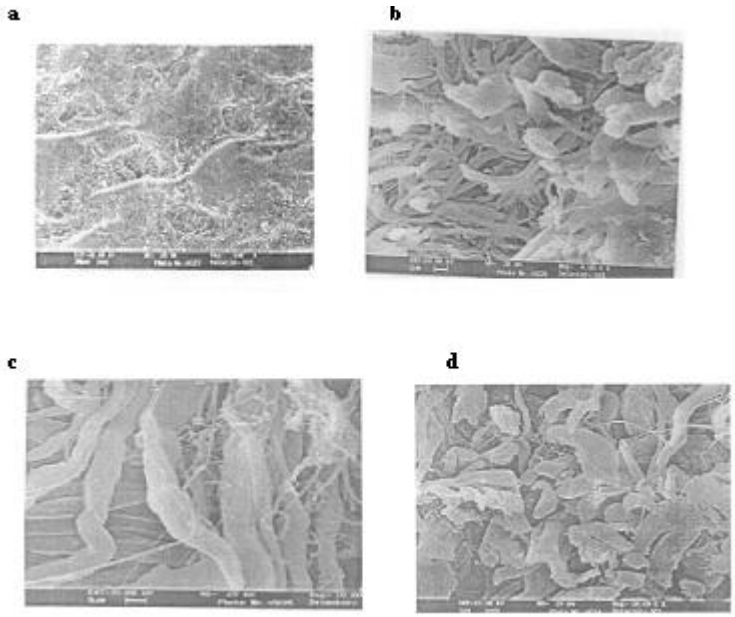

Figure 2: Scanning Electron micrograph of infected leather garment

a & b - Spreading of fungal spores

c & d - Intrusion and invasion of fungal spores