Research on the Effects of TCMTB and N-OITZ based Fungicides used in Leather Industry

by

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ABSTRACT
Currently, TCMTB and N-OITZ are active material of fungicides that are most commonly used in leather industry. The objective of this study was to examine the effectiveness of 2-Thiocyano-methylthiobenzotiazole (TCMTB) and N-octyl-isothiazolinone (N-OITZ) containing commercial fungicides against fungus that grows on leather during pickling and tanning processes. In this study, ASTM D 4576-86 (Reapproved 1991) test standard was applied. During the microbiologic tests, the growth of mold species like ***Aspergillus niger, Alternaria alternata, Penicillium rubrum*** *and* ***Trichoderma viride*** that cause problems in leather industry were investigated against these fungicides. A special attention has been paid in order to purchase the recently produced materials containing active material. The objective of this study was not to examine the MIC values of the commercially available products. Therefore, the amounts indicated in the catalogues were used.

Key words: TCMTB, N-OITZ, wet blue, pickle, fungicide

INTRODUCTION
Fungi are a group of eukaryotic microorganisms distinguished from plants by their lack of chlorophyll, differences in their cell walls, and by the fact that they are not truly multicellular. Together with the bacteria, many fungi play a major role as decomposers serving to bring about the rotting and decay of dead matter, and the recycling of this matter in the environment.[1] This can cause serious damages for pickled pelts and chrome tanned leathers during leather production, since leather is an organic material that consists of many nutrients for the fungi, which acquire the necessary nutrients for growth through absorption. The moulds and unicellular yeasts secrete enzymes into the surrounding environment breaking down (hydrolyze) complex organic compounds into simpler ones. As a result of this extracellular digestion, simpler compounds such as glucose and amino acids can be absorbed.[1]

Fungal growth is influenced by many factors in the environment. Pickled and chrome tanned leather storage under humid conditions, high storage temperature and also the low pH value are the factors causing the growth of mold, besides the organic material of leather.

Both pickled pelts with high amounts of salt and acid, and chrome-tanned leather may be exposed to fungi attack under long storage conditions. 23 different genus were found in chrome tanned leathers: ***Penicillium, Absidia, Acremomium, Aspergillus, Basipetospora, Byssochlomys, Chrysonilia, Cladosporium, Emericella, Eupenicillium, Euratum, Fusarium, Monoscus, Paecilomyces, Mucor, Moniliella, Neosortorya, Phialophora, Scopulariopsis, Stachobotrys, Trichoderma, Trichosporon*** and ***Verticillium).***[2] Researchers have stated that these may cause several damages such as pigmentations in pelts under fungus attack, dyeing and finishing defects in leathers, and fat acid spew on leather surface when fats are decompsed.[3,4,5,6] They may also generate bad smell.

Some researchers noted that fungus growth is inhibited when highly acidic pickle baths are used, and therefore the application of fungicides is not necessary.[7] However, in spite of this inhibition, acid hydrolyze occurs in leather under these conditions, and low acid use in pickled pelts is not recommended. pH level measuring between 1.4 and 1.6 in leathers inhibits fungus growth, yet hydrolyzes in leather proteins may cause pH increase, which provides a more ideal environment for the fungi.[3] According to the general opinion, fungicide use is necessary in pickled and wet blue leathers that require long storage periods.

Currently, products that contain active materials of 2-Thiocyano-methylthiobenzotiazole (TCMTB) and N-octyl-isothiazolinone (N-OITZ) are widely used in the protection of leather against fungi. On the other hand, fungi problem is still observed in some tanneries despite fungicide use. Thus, these two products that contain active materials were treated with pickled and chrome-tanned leathers in active doses recommended by the manufacturer, and the growth of different fungi in leather was examined in accordance with the standards.

Materials and Methods

Materials

Raw skin

In this study, dry salted Turkish domestic sheep skins were used in order to obtain pickled and chrome-tanned leather.

Fungicide

In the experiments, two different fungicides were used: one of them was N-octyl-isothiazolinone (N-OITZ), while the other one was 2-Thiocyano-methylthiobenzotiazole (TCMTB) based. The objective of this study was not to examine the MIC values of the commercially available products. Therefore, the amounts indicated in the catalogues were used after the necessary contacts with the experts of the companies were made, and two different materials were selected for the study. A special attention has been paid in order to purchase the recently produced materials containing active material.

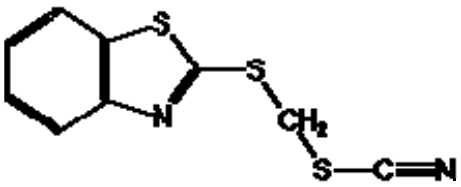

Figure 1:The chemical structure of TCMTB[8]

Molds

Species of mold used in this study are ***Aspergillus niger, Alternaria alternata*** **(NRRL 10593),** ***Penicillium rubrum*** *and* ***Trichoderma viride*****(NRRL 1608).**

Media

Malt Extract Agar (M.E.A)(Merk) was used in keeping stocked cultures and obtaining fresh cultures in the experiments.

Methods

Obtaining the Leather Samples Used in the Experiment

Raw skins were processed so as to obtain two different groups. One group was processed in order to obtain pickled pelts and the other group was processed for obtaining chrome-tanned leathers, which were the main materials of the study. For all leather production, a single standard garment leather processing method was applied.

The group of pickling pelts was obtained during preserved pickling process. The features of the pelts are as follows:

1- Preserved pickled pelt without fungicide (pH:1.5)
2-Preserved pickled pelt obtained by using N-octyl-isothiazolinone (N-OITZ)-based fungicide (0.02%, the amount advised by the chemical company)
3- Preserved pickled pelt obtained by using 2-Thiocyano-methylthiobenzotiazole (TCMTB) based fungicide (0.04 %, the amount advised by the chemical company)
The second group of the leathers, which would be chrome-tanned, was primarily processed through a normal pickling process (pH:3).

4- Chrome-tanned leather without fungicide.
5- Chrome-tanned leather obtained by using N-octyl-isothiazolinone (N-OITZ) based fungicide (0.02 %, the amount advised by the chemical company)
6- Chrome-tanned leather obtained by using 2-Thiocyano-methylthiobenzotiazole (TCMTB based fungicide (0.04 %, the amount advised by the chemical company)
The leather samples used in the experiments were cut in a sterile room under sterile conditions. The laboratory punch was used to punch 3 circular specimens, measuring 3 cm in diameter, out of the leather samples to be tested. All equipments and tools were cleaned with ethanol and were flamed before and after changing samples. The leathers were processed with essential oil or biocide for 4 hours after being cut. Every trial was duplicated and repeated twice.

Activating Mold Species:

Mold species used in the experiments were horizontally prepared and separately incubated in tubes with M.E.A., and fresh cultures were obtained through incubation at 27 ^{o}C for a week.

Microbiological Test Standards:

ASTM D 4576-86 (Reapproved 1991) microbiological test method is used for mold growth resistance of wet blue and other leather samples. In this study, this test standard was appllied.[9] Specimens of the leather were placed in petri-dishes on inoculated nutrient agar-medium and incubated for four weeks. Following this period, the specimens were detected and evaluated respectively in terms of the mold growth.

After 7, 10, 14, 17, 21, 24 and 28 days of incubation periods, the formation of an inhibition zone around the specimens were visually assessed using the following rating scale:

Growth	Assessment	Rating
Specimen with growth	Inadequate	1
Growth on edge of specimen	Inadequate [with note: growth on specimen in (mm)]	2
Growth on cut edges No inhibition zone, no growth on the sample	Still good (limit of efficacy)	2-3
Specimen free of growth; Visible inhibition zone	Good [with note: size of inhibition zone in (mm)]	3

RESULTS AND DISCUSSION

The results of experiments are reported in tables. Accordingly, it was observed that neither TCMTB nor N-OITZ that contain active materials could provide adequate protection against ***A. niger*** and ***T. viride***. The protection of N-OITZ for pickled pelts against ***P. rubrum*** was also inadequate.

TABLE I- Inhibition zone of *Trichoderma viride* **on pickled pelts**

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	1	1	1	1	1	1	1
TCMTB	0,04	1	1	1	1	1	1	1
Pickled pelts (none)	-	1	1	1	1	1	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE II- Inhibition zone of *Aspergillus niger* **on pickled pelts**

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	2 (10 mm)	2 (10 mm)	2 (13 mm)	1	1	1	1
TCMTB	0,04	2 (13 mm)	2 (13 mm)	2 (14 mm)	1	1	1	1
Pickled pelts (none)	-	1	1	1	1	1	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE III- Inhibition zone of *Penicillium rubrum* on pickled pelts

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	3*	3*	3	2-3	2-3	1	1
TCMTB	0,04	3*	3*	3*	3*	3*	3**	3**
Pickled pelts (none)	-	3**	2-3	1	1	1	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE IV- Inhibition zone of *Alternaria alternata* on pickled pelts

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	3*	3*	3*	3*	3 (35 mm)	2-3	2-3
TCMTB	0,04	3**	3**	3**	3 (45 mm)	3 (35 mm)	3 (32 mm)	2-3
Pickled pelts (none)	-	2-3	2-3	2 (4 mm)	2 (15 mm)	2 (15 mm)	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE V- Inhibition zone of *Trichoderma viride* on wet blues

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	2 (7.5 mm)	2 (7.5 mm)	2 (10 mm)	2 (14 mm)	2 (14 mm)	2 (14 mm)	1
TCMTB	0,04	2 (1 mm)	2 (10 mm)	2 (10 mm)	2 (13 mm)	2 (14 mm)	2 (14 mm)	1
Wet blue (none)	-	1	1	1	1	1	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE VI- Inhibition zone of *Aspergillus niger* on wet blues

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	2-3	2 (4 mm)	2 (10 mm)	1	1	1	1
TCMTB	0,04	2 (3 mm)	2 (5 mm)	2 (5 mm)	1	1	1	1
Wet Blue (none)	-	2 (2 mm)	2 (5 mm)	2 (10 mm)	2 (12 mm)	1	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE VII- Inhibition zone of *Penicillium rubrum* on wet blues

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	3*	3*	3*	3*	3 *	3**	3**
TCMTB	0,04	3*	3*	3*	3*	3**	3**	3**
Wet Blue (none)	-	3**	3	3	3	3	3	3

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

TABLE VIII- Inhibition zone of *Alternaria alternata* **on wet blues**

EXPERIMENTS		Number of Days						
Biocides	%	**7**	**10**	**14**	**17**	**21**	**24**	**28**
N-OITZ	0,02	3*	3*	3*	3*	3 (40 mm)	2-3	2-3
TCMTB	0,04	3*	3*	3*	3*	3*	3**	2-3
Wet blue (none)	-	3**	3	2 (4 mm)	2 (4 mm)	2 (10 mm)	1	1

*:no fungal growth on petri dishes **: Small growth near the edges of petri dishes

During the experiments with ***T.viride***, it was detected that TCMTB and N-OITZ based fungicides were not effective enough on this fungi. Both pickled and wet blue leathers that contain these fungicides were covered with growth in a short period of time. The same situation was also observed for ***Aspergillus niger.*** Kennedy reported that there are two types of resistance: inherent and acquired. For example, the mould ***T.viride*** has inherent resistance against TCMTB.[10] This explanation supported the results of this study.

When the trials on pickled and chrome-tanned leathers are examined, it is possible to say that the resistance of chrome-tanned leather against fungi is higher. Karaboz (2001) has reported that heavy metals especially like chrome has oligodynamic effect, which in turn inhibits fungus growth.[11] Yet, according to the data, chrome-tanning has not totally protected leathers against fungus growth. It is likely to say that chrome generally inhibits ***P. rubrum*** growth, and accordingly, chrome alone provided adequate protection against this fungus in the trials even though no additional fungicide was used.

When all the test fungi behaviour against antifungal agent was observed, it was found that each one of them displayed different attitudes. ***Trichoderma viride*** and ***Aspergillus niger*** were generally more resistant, whereas ***P. rubrum*** and ***A. alternata*** were more sensitive. Apparently, the material and dose that is effective on a fungus may not be effectual on another. Therefore, if a mold problem is observed in a tannery, the first thing to be done is to find out the type of the mold; so that it will be possible to detect the most effectual material against this particular type of mold and use the appropriate fungicide. Otherwise, it will be inevitable for the company to lose money and time.

According to the data obtained from the research, the failure of these fungicides containing active materials to provide adequate protection may cause serious problems. Researchers have noted that as a result of insufficient biocide use, microbes develop resistance against these biocides and new microorganism types emerge.[11] Using these fungicides will be unnecessary when microorganisms develop resistance. In this case, different methods may be recommended, such as increasing the proportions, or mixing them with biocides containing different active materials. Researchers have reported that leather industry needs new economic and ecologic biocides that are anti-cancinogen, anti-mutagenic and sensitive to the environment and human health, as a protection against the resistance of microorganisms and emergence of new types.[12]

CONCLUSION

At the end of the trials, it was observed that pickled pelts and chrome-tanned leathers alone could not be effectual against all fungi. Although N-OITZ and TCMTB commercial fungicides that contain active materials were used in effective doses advised by the companies, they were detected to fail in providing adequate protection during test period. It

would be appropriate to examine whether the dose of these fungicides against fungi was insufficient for protection, or whether these fungi has resistance against these active materials, and other factors that may be efficient for effective protection should be investigated. At this point, companies that sell these materials are suggested to continue their research on adequate protection of leathers by their products, and reconsider their recommended doses.

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