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A COMPARATIVE STUDY ON THE CONCENTRATIONS OF TCMTB BASED FUNGICIDES IN LEATHER AND THEIR EFFECT ON MOULD RESISTANCE

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Abstract: *TCMTB (2-(thiocyanomethylthio)-benzothiazole), as a broad spectrum antifungal agent, has found a wide spread usage in processing of pickled, wet-blue and crust leathers and became one of the most used fungicides in leather although it suffered some drawbacks related to its toxicity and handling hazards. Monitoring TCMTB usage and the amounts in leather while keeping the antifungal effectiveness has become a necessity. In this study; 5 different TCMTB based fungicides with similar concentrations sold under different trade names were applied to wet-blue sheep skins. Then the leathers and baths were evaluated for their TCMTB content by using UV Spectrophotometer and HPLC methods to determine the concentration of the active content in fungicides and residue in leather. Meanwhile the effect of the biocides on the fungal resistance of leathers was tested by the use of two methods: tropical chamber and ASTM D4576.*

Key words: TCMTB, Biocide, Fungicide, HPLC, UV Spectrophotometer, fungal resistance

1. INTRODUCTION

As a result of the hydrolytic degradation caused by the mould grow on leather, it can cause irregularities in dyeing and finishing processes, and in the later stages, more serious and irreversible faults on the grain, and may affect the various physical properties of the leather negatively. When appropriate conditions come into existence, mould may also develop in the final product, causing unpleasant appearance and even odours. Although precautions taken for storage conditions can slow down or prevent mould growth, the precise solution can be achieved by using commercial preparations containing active substances called antifungal agents or fungicides.

Some of the earliest products to be used as fungicides were organo mercury compounds (eg. phenyl mercuric acetate aka PMA) and chlorinated phenols (eg. penta chloro phenol aka PCP). These products were effective in degradation of fungi but they were also very toxic to other living organisms, including humans. The use of organo mercury compounds and chlorinated phenols eventually became restricted, starting in the 1970s [1].

Nowadays, only a few fungicides dominate the leather industry usage. The big four fungicides are commonly known by their abbreviations, for example, PCMC (para-chlor-meta-cresol), OIT (2-n-octylisothiazolin-3-one), OPP (ortho-phenylphenol), TCMTB (2-(thiocyano-methylthio) benzothiazole) [2]. These fungicides are also hold under microscope for their toxicity and hazards. The Environmental Protection Agency (EPA) has reviewed risk assessments for

TCMTB and the Reregistration Eligibility Decision (RED) for TCMTB was approved on August 1, 2006 [3].

Ecolabelling is a worldwide voluntary or mandatory labelling system for consumer products, designed to help costumers to select and encourage manufacturers to make products with low environmental impact [4]. The Ecolabel, "Der blaue Engel" (the blue angel), gives recommendations for allowable limits in leather of the active fungicide components: PCMC < 300 mg/kg, OIT < 100 mg/kg, OPP < 500 mg/kg and TCMTB < 500 mg/kg [5].

In this study, the determination and comparison of TCMTB amounts bound in the leather and remaining in the process bath by using UV spectrophotometry and HPLC methods were investigated by using five different TCMTB based commercial fungicides with same active matter. The fungal resistance of the leathers was also evaluated with tropical chamber and ASTM D4575 test method.

2. MATERIAL AND METHOD

2.1 Material

Three wet-blue domestic sheep skins processed without any fungicides were used as leather samples. Five different fungicides that have 30% TCMTB were suplied from the market and coded as T1, T2, T3, T4, T5.

2.2 Method

50 pieces of 7 cm x 11 cm size were cut from various places of wet-blue leathers and separated in groups of 10 for each fungicide. The weight of each group was adjusted to 150±0.1 g.

1 gram of fungicide was added to 1000 ml water. 1/1000 fungicide solutions were applied to the leather samples in 2000 ml flasks for 2 hours at 30°C in a shaking incubator at 150 rpm.

2.2.1 Determination of exhaustion values by UV Spectrophotometer

The leather samples processed with fungicides were ground after drying and the obtained leather powders were washed in distilled water for 6 hours in an orbital shaker. Relative exhaustion values were found by determining the ratio of fungicide removed by washing and comparing the with the stock fungicide solutions by using UV spectrophotometer.

The amount of TCMTB (%) removed by washing (W%) and remained in the leather (B%) were calculated using the following equations (1) and (2).

$$W\% = (Abs.E / Abs.I) \times 100 \quad \textbf{(1)}$$

$$B\% = 100 - W \quad \textbf{(2)}$$

W	The amount of removal by washing in %
Abs.E	Peak absorbance value for washing solution
Abs.I	Peak absorbance value for Stock Solution
B	Binding amount in%

2.2.2 Determination of TCMTB content in leather by HPLC

Leather samples taken according to ISO 2418 were ground according to ISO 4044. 1±0.001 gr of ground leather was weighed and placed in a 100ml beaker, and then 20ml acetonitrile was added. The extraction was performed at room temperature for 1 hour in 80% power ultrasonic bath. Following the extraction, the solution was filtered to for the use of HPLC. HPLC allows the rapid,

sensitive and highly specific determination of fungicide preservatives in leather [6].

CC250 / 4 nucleosil 100-5 C18 HD separator column and appropriate precolumn were used for the HPLC analysis. After isocratic with 60% acetonitrile at the flow rate of 0.8 ml/min, at 30°C column temperature for the first 6 minutes, then 99.7% TCMTB (in acetonitrile) was calibrated in 95% pure water for 9 minutes with 0.02ml injection volume, at UV detection frequency of 275 nm and measurements were conducted under the same conditions.

2.2.3 Standard Test Method for Mould Growth Resistance of Leather - ASTM D4576

The ASTM D4576 method [7] stipulates the using of a series of leather samples checking both grain and flesh side, having the surface of 1 $inch^2$ each inoculated with *Aspergillus niger* and examined after 3, 7 and 14 days. In our study the interval of time was extended to 21 days to get more distinguishable results. The test samples were placed in the center of Petri vessels and then the growing medium (*potato dextrose agar*-PDA), was filled up to the upper level of leather samples. The Petri vessel was incubated for three weeks at the temperature of 27°C. Visual assessment was performed according to the micelle percent on the leather surface at 3, 7, 14 and 21 days. Assessment marks were given depending on this percent, as follows: (0)- mould absent on the surface of sample, (0.5)- less than 12% of sample surface is covered with micelle, (1)- 25% of sample surface is covered with micelle, (2)- 50% of sample surface is covered with micelle, (3)- 75% of sample surface is covered with micelle, (4)- 100% of sample surface is covered with micelle

2.2.4 Tropical Chamber Test

Tropical Chamber test is based on ASTM D3273-00 [8] test method and performed in an insulated cabin whose internal environment is kept at 95-100% humidity and 27-30°C temperature for 4 weeks (28 days). The tropical chamber is infused with the spores of various types of fungi, which are frequently observed in leather and with the help of the air circulation, ideal humidity and temperature these spores affect the leather much faster than normal environment. The evaluation is made by scoring the % surface area covered with mould over 100. Samples scoring 20 and below at the end of each week are considered to have successfully completed the test. Samples showing mould growth below the limit value at the end of the 4^{th} week are considered to have long-lasting mould resistance. Week 3 refers to medium-term and week 2 indicates short-term mould resistance.

In our study, leather samples cut in size of 7 cm x 10 cm were placed on hangers in the cabin. The chamber was infused with *Aspergillus niger* spores and the samples were examined every week. Besides for each test, a piece of leather without fungicides (blank sample) was hung to check if the tropical chamber was working by getting over 20 points at the end of the first week. The mould growth was evaluated for 8 weeks to get distinguishable results for our study.

3. RESULTS AND DISCUSSIONS

When the results of washing and binding ratios of TCMTB based fungicides are examined, it can be seen that there are considerable differences between the binding ratios of the fungicides which have the same benzothiazole active substance (Table 1). The sample of T2 showed the best binding capacity while the sample of T5 had the lowest value. The degree of binding between T3 and T2 samples were found similar. The differences in the binding degree of the samples indicate that the amount of TCMTB bound to leather is affected by other ingredients in the composition of the product.

When the data related to determination of TCMTB content in the leather samples is evaluated, the amount of TCMTB found in the leather sample treated with T3 was found higher than

the others (Table 2). The sample of T5 was the leather with the least TCMTB content. T3 and T2 samples showed similar values.

Table 1 *Washing and binding ratios of TCMTB based fungicides*

Fungicide	Measurement (nm)	Initial Abs.	Final Abs.	Washing Degree (%)	Binding Degree (%)
T1	221	0.79	0.23	28.49	71.51
T2	218.4	1.58	0.34	21.49	78.54
T3	221	1.12	0.25	22.01	77.99
T4	218.2	0.95	0.22	23.68	76.32
T5	221	0.28	0.12	42.84	57.16

Table 2 *The TCMTB contents of the leathers determined by HPLC*

Fungicide	TCMTB amount in Leather Sample (mg/kg, HPLC)	TCMTB (g/Kg)	TCMTB (%)
T1	2635.56	276.40	27.64
T2	3195.65	305.16	30.51
T3	3412.63	328.19	32.82
T4	1732.31	170.24	17.02
T5	1566.05	205.48	20.55

When the mould growth resistance of the samples was tested according to ASTM D4576, it can be seen that all fungicides can provide sufficient antifungal protection against *Aspergillus niger*, graded as "0", at the end of the 21st day. This result was not included in the table because it would be better to give the inhibition zone diameters to compare the effectiveness of the fungicides (Table 3). When evaluated according to the average preserved diameters (Table 3), better fungicidal performances are listed as T1, T3, T2, T4 and T5 in descending order. While T3 and T4 leather samples performed better antifungal activity on the suede side, T1 and T2 had higher antifungal performance on the grain side. T5 sample provided the least protection in both suede and grain side compared to other samples.

The fact that these fungicides, which are produced using the same active substance as benzothiazole, showed different performances on the grain and suede sides of the leathers indicates that the differences that may occur in the auxiliary ingredients used for dissolution, emulsion stability of TCMTB and also the penetration of the fungicide to leather may lead to different results in practice.

Table 3 *Mould Growth Resistance of the Leathers - ASTM D4576*

Fungicide	Mould Resistance Assessment							
	Day 3*		Day 7*		Day 14*		Day 21*	
	Grain Side	Flesh Side	Grain Side	Flesh Side	Grain Side	Flesh Side	Grain Side	Flesh Side
T1	4.27	4.00	4.22	3.97	4.27	4.07	4.27	4.03
T2	4.55	4.10	4.25	3.83	4.22	3.40	4.25	3.40
T3	4.34	4.60	4.12	4.30	4.10	4.10	4.03	4.10
T4	4.02	4.17	3.79	4.07	3.47	4.07	3.47	4.07
T5	3.75	3.63	3.53	3.27	3.22	2.97	3.33	3.03
Blank	2.9		2.27		2.3		2.2	

* Inhibition zone diameter in cm.

According to the results of the first four-week tropical circle test, all leather samples showed that they had long-term mould resistance by scoring below 20 at the end of the 4th week. Starting from the 5th week, an increase in mould growth on the leather samples shows that the fungicide applications of the study were found compatible with the ideal usage rates in real tannery conditions. At the end of the 6th week, T4 fungicide, which remained from the tropical circle test with 30 points, showed the lowest performance among the 5 fungicides applied. The T5 fungicide remained from the test at the end of the 7th week and the T1 fungicide at the end of the 8th week. T2 and T3 fungicides, which scored 20 or less in the tropical chamber test at the end of the 8th week, showed that they provided better protection than the other fungicides. T2 fungicide was slightly more successful than T3 fungicide with an average of 15. (Table 4)

Table 4 *Mould Growth Resistance of Leathers according to Tropical Chamber test*

Fungicide	Mould Resistance Assessment							
	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
T1	0	0	0	0	0	5	15	30
T2	0	0	0	0	0	5	15	15
T3	0	0	0	0	0	5	10	20
T4	0	0	0	5	15	30	40	45
T5	0	0	0	5	10	20	35	50
Blank	40	85	100	100	100	100	100	100

Table 5 gives us opportunity to summarize all findings and make a comparison with TCMTB content of fungicide samples, their binding percentage, bound TCMTB in leather and mould resistance results. According to ASTM D4576 and tropical chamber test results, all fungicides were able to provide adequate protection to the leathers during the standard testing time. In the extended process, the development of mould was started to be observed in leather samples with less TCMTB content. It was concluded that mould resistance is directly relevant to the bound TCMTB in leather. However, the binding % is not always related to the concentration of fungicide as seen T3 and T4. The findings indicate that, while evaluating the performance of fungicides, their ability to bind TCMTB to the leather is important as well as their TCMTB content. Another finding was that TCMTB concentrations of fungicide samples varied in a wide range although they are marketed as similar products. This is another point that consumers should take into consideration.

Table 5: *Comparison of fungal resistance and TCMTB contents*

Fungicide	**TCMTB (%)**	**Binding (%)**	**TCMTB in leather (mg/kg leather)** (HPLC)	**Avg. inhibition zone diameter (cm)** (ASTM D4576)	**Avg. area covered with mould (%)** (Tropical Chamber)
T1	27.64	71.51	2635.56	4.19	30
T2	30.52	78.54	3195.65	4.00	15
T3	32.82	77.99	3412.63	4.05	20
T4	17.02	76.32	1732.31	3.68	45
T5	20.55	57.16	1566.05	3.23	50
Blank	-	-	-	2.20	100

4. CONCLUSIONS

Among the fungicides used in the leather industry, TCMTB has come to the fore with its wide spectrum and compatibility with operating conditions and has found wide usage. It is produced

and marketed under different names by many manufacturers. It is seen that these products, each of which have the same active ingredient and produced with the claim of providing the best protection, are different in many ways and can result different mould resistance to leathers. These differences should be known by the user; arranging the process in accordance with the product character will prevent unnecessary consumption and ensure optimum antifungal protection.

In this study, the binding ability of TCMTB to leather, which is one of the product characteristics that should be considered during the use of fungicide, was determined for each product, and the results were examined comparatively with 2 different antifungal resistance tests. According to the results, TCMTB concentrations for each fungicide, TCMTB binding percentages to the leather, TCMTB amounts in the leather samples and the relationship between this TCMTB amount and the fungal resistance of the leather were revealed. The results showed that the amount of use and product efficiency for each fungicide can be optimally utilized in industrial conditions.

As other fungucides, TCMTB based fungucides are also put under the scope for their environmental impacts. Limitations to their usage and concentrations in leather are discussed. In order to continue using TCMTB-based fungicides in the leather industry, studies on optimizing the usage amounts and developing fungicides with high TCMTB binding ability should be developed.

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