ALTERNATIVE FUNGICIDES FOR THE LEATHER INDUSTRY. DIMPTS and IPBC

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ABSTRACT

Leather industry has a continuous need to adapt their processes to alternative technologies with less environmental impact. The use of fungicides is an essential element, and environmental legislation becoming stricter, leading to the search for new fungicides systems that comply with that law.

The fungicidal capacity of two alternative compounds, diiodometil p-tolylsulfone DIMPTS, 3-iodo-2-propynyl butylcarbamate IPBC, was compared to 2-(thiocianometilthio)-1,3-benzothiazole TCMTB, one of the conventional fungicides in tannery. This fungicidal capacity was evaluated against different strains of fungi in different processes. Different amounts of fungicides were applied in the chrome tanning process and a fatliquoring process of hides tanned with vegetable extracts. Further studies consisted in a microbiological control samples inoculated with fungi common in tannery, determination of the fungicide content on the hides, total and stratigraphic, and a toxicity study of process wastewater.

The effectiveness of DIMPTS and IPBC, verified in previous works, is confirmed when different amounts of fungicide are applied. Consequently, the amount of each fungicide against different strains of fungi can be optimized.

The stratigraphic distribution in different strains of leather (wet blue and vegetable) is also different. The toxicity of wastewater was lower in the case of the alternative fungicides with respect to TCMTB.

Keywords alternative fungicides, strains of fungi, wet blue, vegetable leather, toxicity

1. INTRODUCTION

In leather manufacturing, tanned and untanned leather are threatened of being attacked by a variety of microorganism. Tannery processes offer many possibilities for microbial growth: processing of fresh hides, use of enzymes, fat emulsions and use of natural products for tanning and finishing operations. Other factors such as prolonged storage and transport of hides in wet-blue or pickled state increase the chances of microbial growth.

Hides subjected to production processes such as pickling, tanning, dyeing and fatliquoring are susceptible to fungal attack. For these hides the addition of an efficient fungicide to impede fungal growth is necessary. The search for effective alternative fungicides is the aim of this work.

Hides attacked by fungi show permanent stains and damage in the physical properties because of collagen degradation. The optimal conditions for the growth of a large number of fungal strains are: a pH range from neutral to slightly acidic (3-6), a temperature of 25 ºC and a relative humidity between 12 and 15 %. Wet-blue leather provides the optimal conditions for fungal growth: storage temperature, acid pH and presence of water, proteins and fats. Fungal spores survive in dry conditions and develop again when optimal conditions are recovered (1). According to Hauber (2), the following requirements for a compound to be effective as a fungicide are necessary: optimal activity against a large number of fungi, compatibility with hides and

chemicals used in the tanning process, effectiveness at acidic pH, stability in front of temperature and UV light, low water solubility, low toxicity in humans and economically and environmentally acceptable.

The 2-(thiocyanomethyl) – 1,3 – benzothiazole (TCMTB) was developed as an innovative fungicide into the 70s and still persists today. But the increasingly strict environmental legislation makes that alternatives to this chemical are still necessary (3).

Although a large number of compounds meet these requirements to a greater or lesser extent, TCMTB possesses a wider range of application and has achieved the highest acceptance level in the tanning industry. However, its technical limitations and the considerable environmental impact of TCMTB reinforce the need for looking for new fungicides to replace those conventionally used.

In previous studies (4, 5), fungicides commonly used in the leather sector were compared with other compounds proposed as alternative. It was concluded that two of them (diiodomethyl-p-tolylsulfone, DIMPTS, and 3-iodo-2 propynil butyl carbamate, IPBC) provide a satisfactory resistance to wet-blue leather samples treated with these compounds and thus are proposed as a good alternative for the tanning industry. These compounds were selected according to the chemicals notified and registered in the 98/8/EC Directive. To confirm the results obtained, further studies should be addressed with a wider range of strains and under different conditions.

2. AIM OF THE WORK

This work is focussed on the search for more effective fungicides against a broad spectrum of fungi. To be valid alternatives to the chemicals conventionally used in the leather industry, the new chemicals should be of lower toxicity and responsible of a lower environmental impact.

The main objective of this work is to evaluate the capacity of two selected fungicides, diiodomethyl-p-tolylsulfone, DIMPTS, and 3-iodo-2 propynil butyl carbamate, IPBC, (notified and registered in the 98/8/EC Directive) (6, 7) against different strains of fungi and in different situations. The fungicidal capacity of these compounds will be compared with that of 2-(thiocyanomethyl) – 1,3 – benzothiazole (TCMTB), fungicide commonly used in tannery.

In this study, the minimum amount of the selected fungicides to prevent the attack of fungi will be estimated. Different amounts of the fungicides will be added in two different processes: a) hide chrome tanning, b) fatliquoring process of vegetable tanned leather.

A microbiological control of hide inoculated with strains which are common in tannery will be carried out as well as the stratigraphic and total determination of fungicide content in leather.

Strains provided by the Colección Española de Cultivos Tipo (CECT) from Valencia University and strains isolated in tannery will be used in the analysis of fungal growth.

The determination of toxicity of wastewaters in each of the two proposed processes will complement previous studies.

3. EXPERIMENTAL

3.1. Material

The antifungal capacity of two compounds proposed as alternatives was compared to that of a fungicide conventionally used in tannery. Table 1 shows the fungicides studied in this work (all chemicals are notified and registered in the 98/8/EC Directive) (6, 7).

The fungicidal capacity of the selected fungicides was evaluated against the growth of different strains of fungi which are common in tannery: Aspergillus brasilensis (CECT 2088), Trichoderma harzianum (CECT 2423), Alternaria alternata (CECT 2662) and Penicillium funiculosum (CECT 2914), which were submitted by the Colección Española de Cultivos Tipo Spanish Type Culture Collection (CECT). All the strains were received lyophilized, except Alternaria alternate which was received in culture. They were reconstituted in a suitable agar nutrient medium up to the production of viable spores. The remaining three strains were isolated from hide contamination in tanneries and after reconstitution in a suitable nutrient were sent to the CECT for identification. We named

them as QF01, QF02 and LLO1 and they were identified as:

- QF01 – Penicillium spinulosum
- QF02 – Penicillium decumbens
- LL01 – Trichoderma harzianum

A suspension of spores of each strain was prepared with saline solution at 85 %. Thereafter, the counting of spores was carried out under the microscope and by the plate count method to control the amount of spores/ml when they were inoculated.

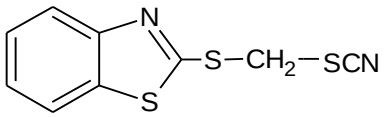
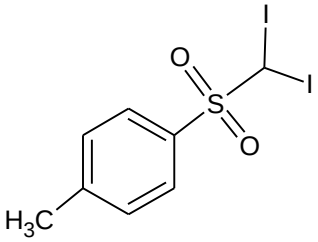
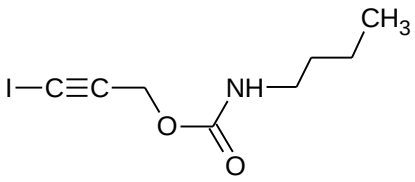
	FUNGICIDE	CHEMICAL STRUCTURE
Fungicide conventionally used in tannery	2-(thiocyanomethyl) – 1,3 – benzothiazole TCMTB (~ 30% active matter)	
Fungicides proposed as alternatives	Diiodomethyl-p-tolylsulfone DIMPTS (~ 40% active matter)	
	3-iodo-2 propynil butyl carbamate IPBC (~ 30% active matter)	

Table 1. Fungicides used in this study

The culture media for developing fungal growth on plate were the following: saboraud dextrose agar and potato dextrose agar.

Figure 1 shows the aspect of the fungi selected for this study.

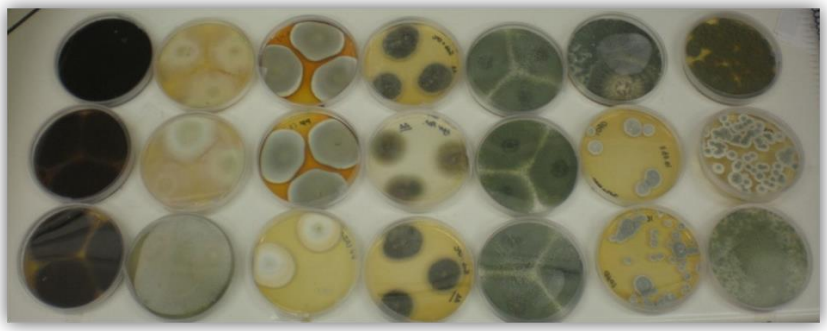


Figure 1. Aspect of the fungi selected on plate (triplicate). From left to right: Aspergillus brasilensis, Trichoderma harzianum, Penicillium funiculosum, Alternaria alternata, Trichoderma harzianum - LL01, Penicillium spinulosum - QF01, Penicillium decumbens - QF02

3.2. Methods

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration (expressed in µg/mL) of an antimicrobial that will inhibit the visible growth of a microorganism after an incubation period. Each fungicide has a

specific MIC for each different microorganism (8).

The minimum inhibitory concentration was determined in accordance with the D 4576-01 ASTM Standard (Test Method for Mould Growth Resistance of Wet Blue). Several dilutions at concentrations ranging from 10240µg/mL to 0.078µg/mL of the fungicides under study were prepared, and the dilution which inhibited the growth of the fungi was determined (9).

The MIC determination of the fungicides selected for this work against the abovementioned fungal strains was performed in previous studies (4, 5). These results are included in this article in order to observe the antifungal capacity of the selected chemicals in front of the strains.

3.3. Application of different offers of the selected fungicides in two different processes: a) hide chrome tanning, b) fatliquoring process of vegetable tanned leather.

French hide (32kg+) was used as the starting material in both processes. Laboratory drums were used in this study.

Before starting the tests, each hide was extracted with acetonitrile to determine by HPLC possible residual fungicides from earlier operations. This was necessary to make sure that the hides did not contain residual fungicides which could interfere with the results.

In both cases, each hide was cut into nine similar samples. In one sample of each process, no any fungicide was added and was kept as control sample. Four different offers (C1 – C4) of the two studied fungicides (DIMPTS and IPBC) were applied to the eight remaining samples, as shown in Table 2.

Sample	Fungicide	Sample	Fungicide
M1	C1 - DIMPTS	M5	C1 - IPBC
M2	C2 - DIMPTS	M6	C2 - IPBC
M3	C3 - DIMPTS	M7	C3 - IPBC
M4	C4 - DIMPTS	M8	C4 - IPBC

Table 2. Treatments with fungicides applied in each process

a) Chrome tanning

Pickled hide, pelt split at 3.3 mm, was used as the starting material. Each sample was tanned in a different drum in accordance with the recipe shown in Table 3.

Percentage on pickle weight	
60% H_2O	
4% sodium chloride	
4% chromium salt 33% basicity	Drum 60’
(X/2) % fungicide	Drum 60’
4% chromium salt 33% basicity	Drum 3h
1% sodium formate	Drum 3h
Rest overnight	
pH control (2,8 – 3)	
1.5% sodium hydrogen carbonate	Drum 90’
pH control (3,5 – 4)	
(X/2) % fungicide	Drum 60’
Drain off	
100% H_2O	Drum 15’
Drain off	

Table 3. Chrome tanning recipe

The different amounts (X) of fungicide added are shown in Table 4.

Sample	Fungicide	Sample	Fungicide	% fungicide
M1	C1 - DIMPTS	M5	C1 - IPBC	0.12
M2	C2 - DIMPTS	M6	C2 - IPBC	0.16
M3	C3 - DIMPTS	M7	C3 - IPBC	0.20
M4	C4 - DIMPTS	M8	C4 - IPBC	0.24

Table 4. Different amounts of fungicide added in the chrome tanning operation.

Two pieces were separated from each tanned sample:

- A wet sample to observe fungal growth on the leather. This sample was kept at 4 ºC
- A dry sample to determine the content of fungicide on the leather. This sample was stored at room temperature.

b) Fatliquoring process of vegetable leather

Pickled hide similar to that employed in the previous study was used as starting material. This pickled hide was tanned with vegetal extracts in accordance with the recipe shown in table 5. Once tanned, the leather was cut into nine similar samples. Each one of them was fatliquored according the recipe shown in Table 6.

Percentage on pelt weight	
4 % mimosa extract	
1 % naphthalene sulphonic dispersant	
0.18 % sodium hydrogen carbonate	
0.26 % sulphated fatliquoring agent	Drum 2h
30 % H_2O (at 35ºC)	
6 % mimosa extract	Drum 2h
10 % mimosa extract	
0.5 % naphthalene sulphonic dispersant	Drum 12h
0.4 % oxalic acid	
0.4 % EDTA (disodium salt)	
50 % H_2O	Drum 1h
Drain off	
200 % H_2O	Drum 20’
Drain off	
Hide discharge	
Drain off	

Table 5. Vegetal tanning recipe

Percentage on drained weight	
70 % H_2O (55ºC)	
4,30 % sulphated fatliquoring agent	
1 % sulphited fatliquoring agent	
X % Fungicide	
0,45 % EDTA (disodium salt)	Drum 60’
0,17% oxalic acid	Rodar 25’
Drain off	
160% H_2O	Rodar 1’
Drain off	

Table 6. Recipe of the fatliquoring process of vegetal leather

The different amounts (X) of fungicide added are shown in Table 7.

Sample	Fungicide	Sample	Fungicide	%fungicide
M1	C1 - DIMPTS	M5	C1 - IPBC	0.02
M2	C2 - DIMPTS	M6	C2 - IPBC	0.04
M3	C3 - DIMPTS	M7	C3 - IPBC	0.06
M4	C4 - DIMPTS	M8	C4 - IPBC	0.08

Tabla 7. Different amounts of fungicide added in the fatliquoring process of vegetal leather

As in the previous study, two pieces were separated from each tanned sample:

- A wet sample to observe fungal growth on the leather. This sample was kept at 4 ºC
- A dry sample to determine the content of fungicide on the leather. This sample was stored at room temperature.

3.4 Control of fungal growth

Control of fungal growth on each leather sample treated with different amounts of DIMPTS and IPBC against three selected strains were performed. Two of these strains were submitted by the CECT and the third was isolated from hide contamination in tannery. The study was carried out for triplicate on sterile plates with potato dextrose agar culture medium in accordance with the ASTM D4576-01 Standard (10). The growth was controlled against a blank sample consisting of a hide of the same characteristics but without the addition of fungicide.

The mould growth resistance of the fungicide treated samples was tested against Aspergillus Brasilensis, Thrichoderma harzianum and QF01 (Penicillium spinulosum). The samples were placed in plates surrounded by the culture medium. After solidification of the agar, one drop of the spore suspension (1x105 spores/ml)of each selected mould was deposited directly on the sample and another drop on the culture medium as shown in Figure 1.

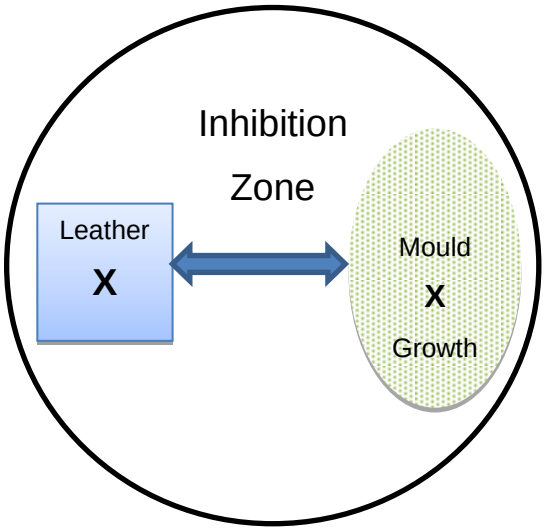


Figure1. Sample with inocolum locations (X), inhibition zone (IZ) provided by the fungicide present on the leather

The plates were stored in a humid atmosphere at 26 ºC and the control of fungal growth was performed weekly against the blank sample up to a maximum of 90 days. In the case of vegetable tanned leather, fungal growth control was performed for 30 days since this type of leather is exposed to potential fungal attack for a maximum of 2 or 3 weeks.

We observed the fungal growth in the treated samples placed in the plates to evaluate the results obtained in this study. According to the ASTM D 4576-01 Standard (10), the report of results of mould growth is rated as a percentage of leather surface covered by mould. In order to be more precise in the results of samples where the growth was 0%, we have introduced another parameter called "inhibition zone" (IZ) (Figure 1) which can be defined as the distance (mm), around the sample, in which growth of the mould is prevented by the protecting effect of the fungicide.

3.5 Determination of fungicide content in leather

3.5.1 Total content of fungicide

Samples treated with the different fungicides were allowed to dry at room temperature. Once dried, they were ground to obtain hide powder. Fungicide content was determined by liquid chromatography in accordance with the ISO/DIS 13365 Standard. (11). In a first step, the treated samples were extracted with a suitable solvent in ultrasounds. Afterwards, the fungicide content in the extract was determined by HPLC. Acetonitrile was used as solvent in all cases.

3.5.2 Stratigraphic determination of fungicide content in leather

Parallel to these studies, a wet-blue tanning process and a fatliquoring process of vegetal leather were performed (following the same formulations as described in Table 3 and Tables 5 and 6, respectively). A common offer of fungicide in these processes was added. Once obtained, both leathers were allowed to dry at room temperature. Afterwards, the dried leathers were split into three layers: grain, intermediate and flesh. Each layer was ground and fungicide content was determined by HPLC. Thus the stratigraphic distribution of each fungicide in leather and for each process was evaluated.

An offer of 0.2 % of the studied fungicides (TCMTB, DIMPTS and IPBC) was added in the wet-blue tanning process whereas an offer of 0.04 % of fungicide was added in the fatliquoring process of vegetal leather

3.6 Toxicity of process wastewaters

Wastewaters of processes performed in the above paragraph were analyzed for toxicity in accordance with the UNE EN ISO 11348-3:2009 Standard by the Microtox method (12). Thus the environmental impact of the alternative fungicides (DIMPTS and IPBC) was compared with that of fungicides conventionally used in the tannery (TCMTB).

The Microtox® system is a bioassay which examines acute toxicity of environmental samples and pure compounds and is based on the decrease of natural bioluminescence of the Vibrio fischeri marine bacterium in the presence of contaminants.

Toxicity is expressed as the concentration of contaminant that causes the diminution of 50 % of the initial luminescence (EC50) (13).

4 RESULTS

4.1 Determination of Minimum Inhibitory Concentration (MICs)

Table 8 shows the results of the MICs of the selected fungicides against the strains of the fungi considered.

	Strains submitted by CECT				Isolated strains		
Fungicide	***Aspergillus brasilensis***	***Trichoderma harzianum***	***Penicillium funiculosum***	***Alternaria alternata***	***QF01***	***QF02***	***LL01***
TCMTB	7.6	7.6	15.3	0.95	61	17	17
DIMPTS	3.8	7.6	1.9	1.07	7.6	15.2	15.2
IPBC	0.8	3.9	1.94	0.79	3.8	3.8	3.8

Table 8. MICs, in μg/mL, of each of the studied fungicides against the fungi assayed

The fungicides proposed as alternatives show an antifungal capacity superior to that of TCMTB not only against strains provided by the CECT but also against strains isolated from the tannery. These strains isolated from the tannery revealed an "acquired" resistance to these compounds. This is probably because these strains resisted the action of conventional fungicides possibly applied in the tanning process.

4.2. Application of fungicides in two different processes.

a) Hide chrome tanning. Control of fungal growth

Table 9 shows the percentage of wet-blue leather surface covered by fungal growth and the inhibition zone for the different offers of fungicide applied. The study was carried out for triplicate and the results are the mean values.

Wet Blue leather		***Aspergillus brasilensis***		***Trichoderma harzianum***		***QF01 - Penicillium spinulosum***	
DIMPTS		**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**
C0	**Control**	100	0	100	0	100	0
C1	**0.12**	0	21.7	0	12.7	0	18.3
C2	**0.16**	0	21	0	12	0	14
C3	**0.20**	0	20	0	15	0	14
C4	**0.24**	0	29	0	13.7	0	18
IPBC		**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**
C0	**Control**	100	0	100	0	100	0
C1	**0.12**	0	26.7	37	0	13.3	6
C2	**0.16**	0	45	0	12.3	0	20
C3	**0.20**	0	39.3	0	22.7	0	32
C4	**0.24**	0	45	0	19.7	0	37.7

Table 9. Results of fungal growth in surface (CS) and Inhibition Zone (IZ) for wet-blue samples, with different offers of fungicide (DIMTPS e IPBC), alter 90 days of control.

If the strain reaches the wet-blue sample, i.e., fungal growth in the sample is observed, there is no inhibition zone. Samples without added fungicide were completely covered by fungi two weeks after spore inoculation. The lowest offer (0.12 %) of DIMPTS was sufficient to inhibit fungal growth on wet-blue samples. In

the case of IPBC, this offer was also sufficient to inhibit the growth of Aspergillus Brasilensis. However, an offer a little bit higher (0.16 %) was necessary against the growth of Trichoderma harzianum and Penicillium spinulosum.

Image 2 is an example of growth control in samples where Trichoderma harzianum was inoculated. The experiment was carried out in triplicate and each column corresponds to a different concentration of IPBC. This image has been chosen since it is clearly observed that different concentrations have different effects against strains. The highest the concentration applied, the highest the protective effect in wet-blue samples. Moreover, IPBC prevented fungi from growing around the samples by creating an efficient inhibition halo.

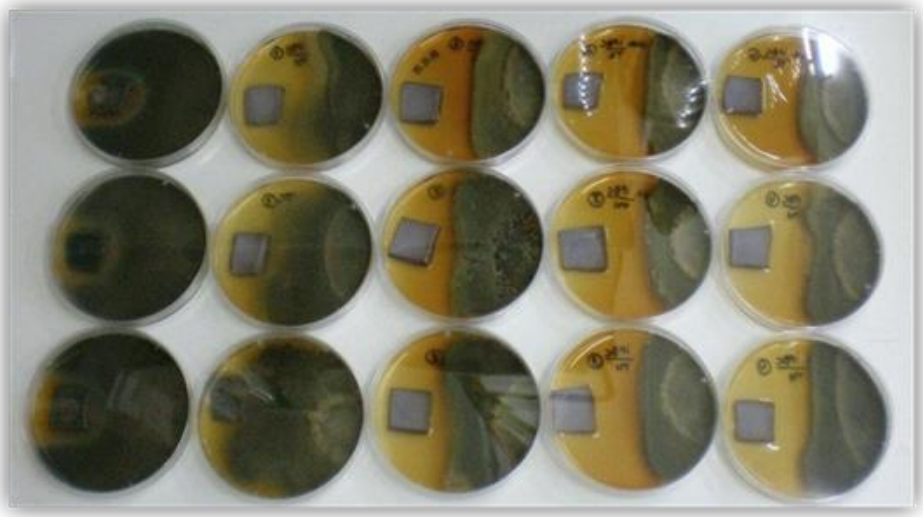


Image 2. Growth of Trichoderma harzianum on wet blue samples alter 90 days of testing. From left to right: Control, 0.12%, 0.16%, 0.20%, 0.24% IPBC

b) Fatliquoring process of vegetable leather. Control of fungal growth

Table 10 shows the percentage of surface of vegetable tanned and fatliquored samples covered by fungal growth and the inhibition zone. In this process, lower concentrations of fungicide were applied since this type of leather is exposed to potential fungal attack for a maximum of 2 or 3 weeks. Fungal growth control was performed for 30 days.

Vegetable leather		*Aspergillus brasilensis*		*Trichoderma harzianum*		*QF01- Penicillium spinulosum*	
DIMPTS		**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**
C0	**Control**	84.3	0	100	0	100	0
C1	**0.02**	0.3	3	12.3	0	0.7	0
C2	**0.04**	0	7	1.7	4.3	0.2	1
C3	**0.06**	0	7.3	0	9.3	0.7	5.7
C4	**0.08**	0	21.3	0	7	0	12.3
IPBC		**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**	**CS (%)**	**IZ (mm)**
C0	**Control**	78.3	0	100	0	100	0
C1	**0.02**	0	10	40	0	0	4
C2	**0.04**	0	14.7	11	0	0	7.7
C3	**0.06**	0	21	6	0	0	11
C4	**0.08**	0	26	1.2	0	0	13.7

Table 10. Results of fungal growth in surface (CS) and Inhibition Zone (IZ) for vegetable tanned and fatliquored samples, after 30 days of control

The results show that for this type of leather smaller quantities of fungicide were sufficient to prevent fungus attack. A 0.06 % of DIMPTS was sufficient to impede the attack of Trichoderm harzianum; for Aspergillus brasilensis, a 0.04% was necessary whereas for Penicillium spinulosum the offer should be increased up to 0,08 %. As far as IPBC is concerned, a 0.02 % of this fungicide was enough to prevent contamination by Aspergillus brasilensis and Penicillium spinulosum, but an offer higher than 0.08 % was necessary to avoid the attack of Trichoderma harzianum for one month.

The effect exerted by different offers of IPBC applied on vegetable and fatliquored leather against the attack of Penicillium spinulosum (QF01) after 30 days of control is presented in Image 3.

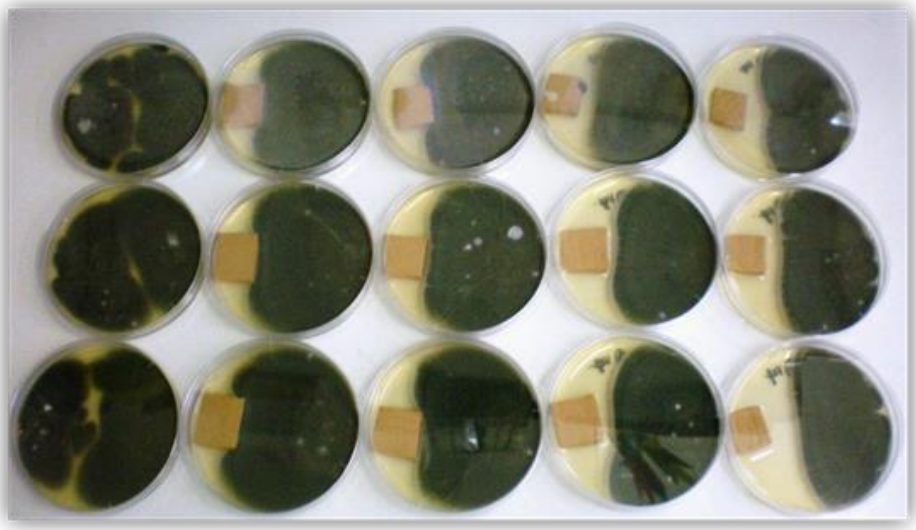


Image 3. Growth of QF01 (Penicillium spinulosum) on vegetable and fatliquored leather, after 30 days of control. From Leith to right: Control, 0.02%, 0.04%, 0.06%, 0.08% IPBC.

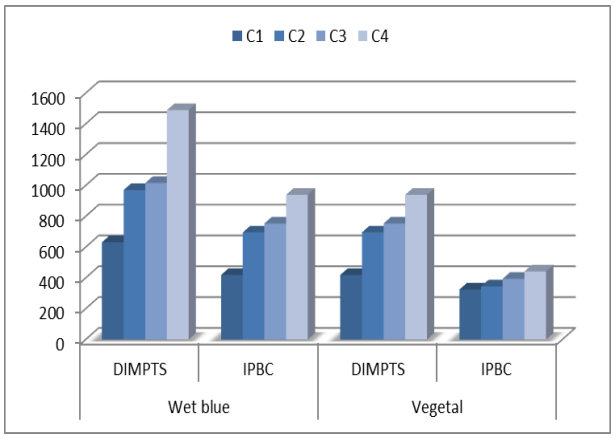
It is more difficult to protect vegetable leathers against fungal growth because vegetable extracts (polphenols combined with carbohydrates) provide fungi with a direct nutrient in the form of simple sugars.

4.3 Determination of fungicide content in leather

4.3.1 Total content of fungicide

The higher the percentage of fungicide added to the process, the greater amount of fungicide found in the leather, although the proportion was not maintained between samples. Possibly this is due to that hide is an irregular matrix and the fungicide is not deposited uniformly.

Graph 1 shows the total content of fungicide in leather as a function of the different offers applied in the two processes and for the two fungicides considered.



Graph 1. Total content of fungicide in leather

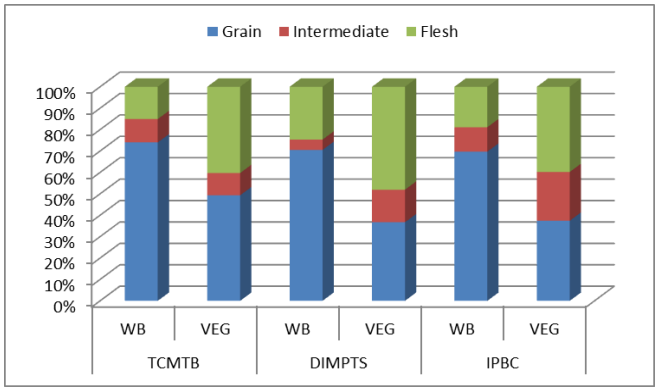
4.3.2 Stratigraphic determination of fungicide content in leather

In accordance with Hauber (14), a normal stratigraphic distribution of fungicides in a hide is as follows: 60-70 % in grain; 10-20 % in the intermediate layer and 25-30 % in flesh. By observing the results obtained (Table 11 and Graph 2), we can state that, in the case of wet-blue tanning process, almost all products tested met these criteria. However, the fungicide content in the outer layers (grain and flesh) was very similar whereas the content

was a little bit lower in the intermediate layer when considering the results for the fatliquoring process of vegetable leather.

	TCMTB		DIMPTS		IPBC	
	Wet Blue	Vegetable	Wet Blue	Vegetable	Wet Blue	Vegetable
Grain	74.0	49.3	70.5	36.7	69.7	37.4
Intermediate	10.9	10.4	4.8	15.2	11.4	22.8
Flesh	15.1	40.3	24.7	48.1	18.9	39.8

Table 11. Fungicide content (%) in each layer for the two processes



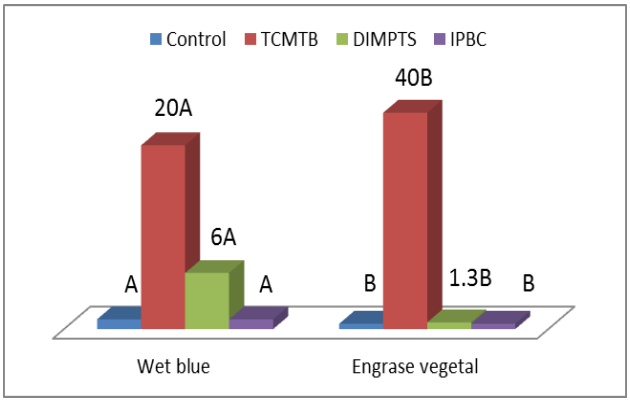
Graph 2. Stratigraphic distribution of fungicide in leather

A distinct stratigraphic distribution of fungicide was observed in the two processes: while wet-blue leather samples followed the Hauber criteria (the highest content (60-70 %) of fungicide was deposited in the grain layer), in the case of vegetable tanned and fatliquored samples, grain and flesh layers had a similar fungicide content (35-50%) and the intermediate layer had the lowest content in (10-20%).

4.4 Determination of toxicity of process wastewaters

The toxicity of a bath without added fungicides (control) was compared to that of wastewaters of processes (wet-blue tanning and fatliquoring of vegetable leather) to which the studied fungicides (TCMTB, DIMPTS e IPBC) were added.

In Graph 3, the first column on the left in each case corresponds to the toxicity of the control bath (without added fungicides). This toxicity was named A and B, respectively. The toxicity of wastewaters of processes with added fungicides is expressed as multiples of the toxicity of the control bath.



Graph 3. Comparison between toxicity of studied processes wastewaters and control bath.

TCMTB showed the highest toxicity, one of the major problems associated to this molecule. The two fungicides proposed as alternative showed a much lower toxicity. The toxicity of wastewaters with IPBC should be underlined since it was similar to that of the control bath (without added fungicides) for the two processes, wet-blue tanning and fatliquoring of vegetable leather.

However, the TCMTB is environmentally unstable. It is stable at the acid pH of wastewaters but gradually degrades at a pH value of 7. The rate of hydrolysis increases rapidly at higher pH values. This means that under the conditions of the wastewater treatment plant, toxicity of TCMTB will be much less important than in wastewater conditions (15).

5 CONCLUSIONS

The fungicides proposed as alternatives, diiodomethyl-p-tolylsulfone, DIMPTS, and 3-iodo-2 propynil butyl carbamate, IPBC, showed a higher antifungal capacity than 2-(thiocyanomethyl) – 1,3 – benzothiazole (TCMTB), fungicide commonly used in tannery not only versus strains provided by the CECT but also in front of strains isolated from tannery. These strains isolated from tannery revealed an enhanced resistance to any new fungicide since they survived the action of commonly used fungicides probably applied in

tannery processes, i.e., these isolated strains posses an "acquired" resistance to these compounds.

The results obtained in this work, together with the conclusions drawn from two previous studies, confirm the capacity of these two alternative fungicides (DIMPTS and IPBC) in the tanning industry.

An offer of 0.2% of fungicide is an amount normally applied in wet-blue tanning. However, the results of this work have demonstrated that lower offers (between 0.12 % and 0.16%) of DIMPTS and IPBC would be sufficient to avoid the attack of the three strains considered in this study. Strains that are common in tannery.

In the case of vegetable and fatliquored leather, an offer from 0.04 % to 0.08% of DIMPTS would be necessary to protect it completely during four weeks. On the contrary, a 0.02% of IPBC would be sufficient to confer adequate protection for a month against two of the three strains considered (Aspergillus brasilensis and Penicillium spinulosum). It should be pointed out that different types of fungi require different amounts of fungicide, as observed from the MICs results.

In relation with the stratigraphic distribution of fungicide on leather, we observe that while in the wet-blue leather both fungicides were distributed approximately a 60-70% in the grain layer, a 10-20% in the intermediate layer and a 25-30% in the flesh layer, results that agree with bibliography, in the case of vegetable leather, the distribution is somewhat different. The two external layers (grain and flesh) contained similar amounts of fungicide (between 35-50%) and the intermediate layer only between 10 and 20%. It is possible that increased protection on the outside of the leather prevented the entry of spores.

The evaluation of the toxicity of process wastewaters confirmed a major problem of 2-(thiocyanomethyl) – 1,3 – benzothiazole (TCMTB) since this chemical showed by far the highest toxicity (between 20 and 40 times the toxicity of the control bath, without added fungicide).IPBC gave rise to the lowest toxicity since the toxicity of its wastewaters coincided with that of the control bath (without added fungicides). DIMPTS toxicity was very much lower than that of TCMTB. DIMPTS toxicity was between 1.3 and 6 times that of the reference. All toxicity values were determined at the final pH of the process and they decrease when pH value reaches a value of 7.

6 ACKNOWLEDGEMENTS

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