2-Amino-6-Benzothiazole Substituted with Methoxy in the Form of Sulfonic Acids, used As Antifungal Product for the Preservation of Wet-Blue Bovine Hides

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Abstract: This paper presents results of a biocidal product derived from 2-amino-benzothiazole-6-methoxy in the form of sulphonic acids, which was synthesized for the purpose of antifungal protection of wet-blue bovine hides. The biocide samples have been offered in proportions of 0.5% and 1.0% to wet-blue bovine hides for antifungal protection and their absorptions in the hide samples were determined by using UV spectra. Wet-blue leather samples have been tested for the antifungal activity against some species of fungi that grows on wet-blue hides: *Aspergillus niger, Penicillium aurantiogriseum, Scopulariopsis brevicaulis.*

Keywords: 2-amino-benzothiazole-6-methoxy sulphonic acids, UV spectra, wet-blue hides, antifungal protection

1 Introduction

Due to extended times in transport and storage, leathers in the wet blue and wet white condition need protection against bacterial and fungal contamination [1]. Reasons of fungal growth are mainly environmental conditions like storage at high temperature and high humidity, long storage times and pH values *(4-6 ph)* [1, 2].

Since fungal spores are easily transferred by air or physical contact, fungi proliferate on pickled hides, vegetable-tanned hides, wet-blues, and finished leather when environmental conditions are favourable and no fungucide is used [3].

Over 20 fungi species are isolated from the leather. However, main fungi species mostly grow on leather are *Aspergillus niger, Trichoderma viride, Penicillium glaucum, Penicillium cyclopium, Paecilomyces varioti, Scopulariopsis brevicaulis* [2].

Fungicides are crucial chemicals for the production and especially for the distribution of leather and leather products. For any biocide to be of value in the tanning industry it should have the following properties: (a) high activity; (b) a broad antimicrobial spectrum; (c) compatibility with leather and with pickling, and chrom- and vegetable-tanning liquors; (d) stability on leather; (e) non-discolouring; (f) environmentally acceptable; (g) low toxicity to humans and other warmblooded animals; and (h) cost effectiveness [4].

Most common active substances for the fungicides in the market are Thiocyanomethylthiobenzothiazole (TCMTB), o-Phenylphenol (OPP), p-Choloro-m-cresole (PCMC),

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n-Octylisothiazolinone (OIT), Methylen-bis-thiocyanate (MBT), Carbendazim (BCM), Diiodomethyltolylsulfone (DIMTS) [1].

TCMTBs which are commonly used in the market, show low water solubility and have no reacting group enabling their binding to the leather, and they are washed off in the subsequent stages in leather processing [5]. Also A. Orlita (2004) mentioned that they are inactivated by alkali and can be affected during basification and they are also preferentially absorbed by fats and thus its effective concentration is lowered. Additionally, the instability of TCMTB, its degradation in alkaline solutions, in high temperature, in the presence of sulphide or when exposed to sunlight is known [6, 7] .

Therefore, this research is aimed at obtaining some biocides with a benzothiazole structure, containing a sulphone group in their molecules that is to be bound ionically to the leather in an acid medium (-NH3+, -SO3-) while the –COO- group in collagen is capped by chromium, achieving thus an antifungal protection in half finished leather products.

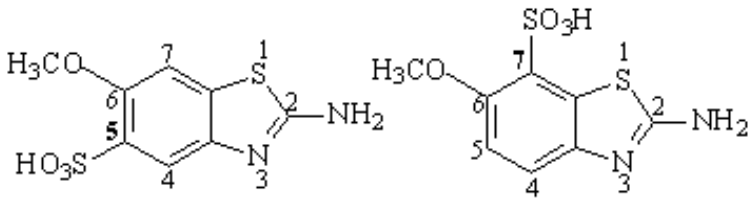
From this point of view, a biocidal product derived from 2-amino-benzothiazole-6-methoxy in the form of sulphonic acids was used in the proportions of 0.5% and 1% for the purpose of antifungal protection of wet-blue bovine hides. Their absorptions were determined by using UV spectra from the process floats. Wet-blue leather samples have been tested for the antifungal activity against some species of fungi that grows on wet-blue hides: *Aspergillus niger, Penicillium aurantiogriseum, Scopulariopsis brevicaulis*.

2 Experimental

2.1 Materials

Wet-blue bovine leather samples were used for testing of the antifungal activity. A limed split bovine hide was used for as the material. It was processed till the wet-blue stage.

Four new fungicide products, by sulphonation of derivatives of 2-amino-benzothiazole-6– substituted with methyl, methoxy, chlorine and nitro were synthesized and methoxy substituted derivates gave best results among them in previous studies [8, 9]. Therefore, 2-aminobenzothiazole-6-methoxy, mixture of the sulphonic acids was used as the antifungal product (Figure 1) for determining antifungal effect on 3 different species of fungi that commonly grow on the leather: *Aspergillus niger, Penicillium aurantiogriseum, Scopulariopsis brevicaulis.* These standard species were obtained from DSMZ, Germany.



2B **2C**

Fig. 1 Chemical Structure of synthesized Antifungal Product

Thiocianic acid, (2-benzothiazolylthio, methyl ester) based commercial fungicide was used for comparison. In addition, one wet-blue bovine leather sample without any protection against fungi was also used as blank sample.

2.2 Methods

Limed split bovine hide was processed as usual to the pickling process. At this step, the new biocide was given in proportions of 0.5 and 1%. A thiocianic acid, (2-benzothiazolylthio, methyl ester) based commercial sample and a wet-blue leather without any precaution were used as blanks.

Before processing and after 30 minutes, the float samples were collected for the UV analysis for determining the exhaustion degree of the biocides from the leather processing floats. In order to achieve the UV spectra, a solution of 1 ml float was diluted with distilled water and the exhaustion of floats has been calculating using the formula below:

Exhaustion degree, GE30 % =(ai - af30) / ai x100

The tanning process was made with 8% basic chromium sulphate at 28ºC in a float of 150% for 8 hours and the basification process was done afterwards. The leather samples were kept in a cool place and prepared for the tests as described below.

For determining the washing degree of biocides, 5g of grinded wet-blue bovine leather samples have been stirred with distilled water at room temperature in a laboratory stirrer for 6 hours and then they filtered. The next day, from the aqueous filtered product the UV spectra has been done, which has been compared with the initial float spectra. From the absorbance ratio for the main peak it has been calculated the unbound biocide percent (Gw%) with the formula below:

Gw, % = (awx 100) / ai

After determining the washing degree, the bounding degree of biocides is calculated using this formula**:** GL , % = 100 – Gw, %

The antifungal activity has performed in conformity with ASTM D 4576-86: 1996 (Standard Test Method for Mold Growth Resistance of Blue Stock, Leather) [10]. The wet-blue leather samples were cut into pieces having the surface of 1 inch^2 and the assays were done in triplicates as described in the method. Additionally, 2 different fungi species named as *Penicillium aurantiogriseum, Scopulariopsis brevicaulis* were used in this study, except from the test organism *Aspergillus niger,* mentioned in the method.

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 vessels were incubated for two weeks at the temperature of 26-30ºC. At 3, 7 and 14 days the petri vessels were checked and evaluated visually according to the assessment as given below.

0	mould absent on the surface of sample
0,5	less then 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

The leather samples in Petri dishes have been also photographed and the inhibition zones were measured at 3, 7 and 14 days incubation.

3 Results and Discussion

UV spectra's of the initial float and the float taken after 30 minutes belonging to the benzothiazole based fungicide and commercial biocide are presented in Figures 2 , 3, and 4 the exhaustions of floats have been calculated from the ratio of the main peaks absorbance.

The exhaustion degrees of the biocide sample were found 55% and 59% respectively as for the proportion of 0.5% and 1% although it is found 65% for the commercial sample.

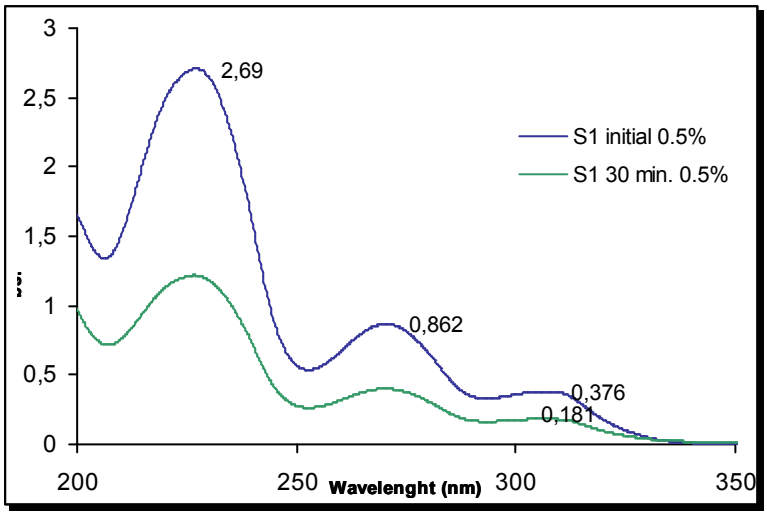


Fig. 2 UV spectra of absorbance of floats for the benzothiazole based fungicide (0.5%)

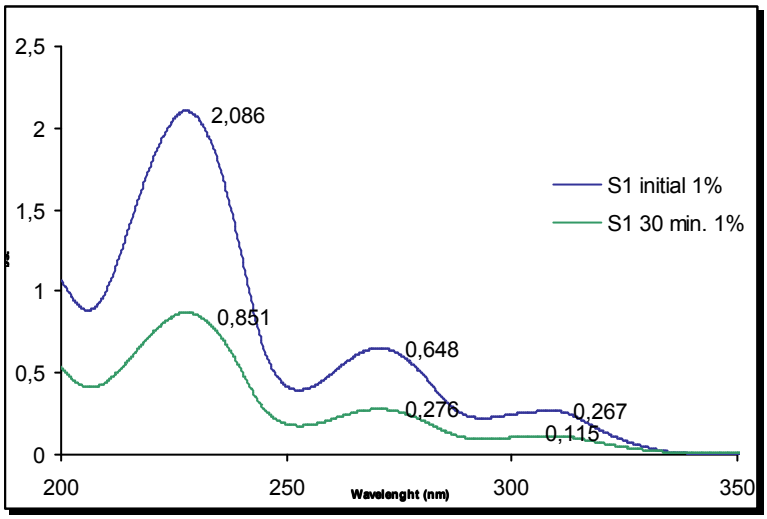


Fig. 3 UV spectra of absorbance of floats for the benzothiazole based fungicide (1%)

Washing degree results of the samples were found 4.2% and 3.8% for the proportions of 0.5% and 1% respectively. But for the commercial sample, this result was found 12.6% and it could be understood from this results that commercial sample could be washed off more easily than the benzothiazole samples.

Besides this, the bounding degree results of the samples were found 95.8% and 96.2% for the proportions of 0.5% and 1% respectively although the result of the commercial sample was found 87.4%.

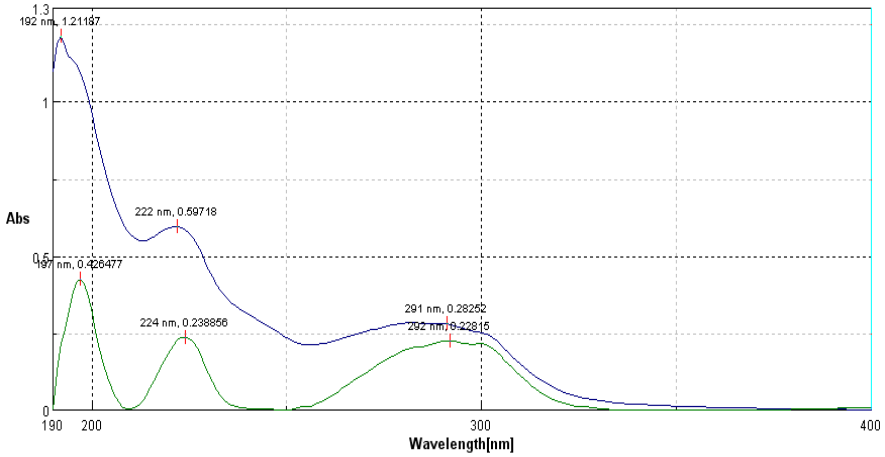


Fig. 4 UV spectra of absorbance of floats for the commercial sample

The 3 day results of ASTM method with *Aspergillus niger* showed that there was no growth for all the samples. But for the 7 and 14 day results, the samples were covered with *A. niger* although the commercial and blank samples have better results.

Tab. 1 Assessment of antifungal activity of wet-blue leathers with *Aspergillus niger*

			Days						Days		
			3	7	14				3	7	14
ASPERGILLUS NIGER	**Sample 0.5%**	Grain Side	0	4	4	ASPERGILLUS NIGER	**Sample 1%**	Grain Side	0	4	4
		Flesh Side	0	4	4			Flesh Side	0	4	4
	C. Fungicide	Grain Side	0	0 – 0.5	1		**C. Fungicide**	Grain Side	0	0 – 0.5	1
		Flesh Side	0	0	0.5			Flesh Side	0	0	0.5
	Blank	Grain Side	0	0	2		**Blank**	Grain Side	0	0	2
		Flesh Side	0	0.5	4			Flesh Side	0	0.5	4

The results of *S. brevicaulis* and *P. aurantiogriseum* showed that there was no growth at the samples at 3, 7 and 14 days. It is understood from the Table 2 and 3 that the benzothiazole based fungicide has better results compared to the ones obtained from *A.niger*s.

Tab. 2 Assessment of antifungal activity of wet-blue leathers with *S. brevicaulis*

			Days						Days		
			3	7	14				3	7	14
S. BREVICAULIS	**Sample**	Grain Side	0	0	0	S. BREVICAULIS	**Sample**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0
	C. Fungicide	Grain Side	0	0	0		**C. Fungicide**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0
	Blank	Grain Side	0	0	0		**Blank**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0

Tab. 3 Assessment of antifungal activity of wet-blue leathers with *P. aurantiogriseum*

			Days						Days		
			3	7	14				3	7	14
P. AURANTIOGRISEUM	**Sample 0.5%**	Grain Side	0	0	0	P. AURANTIOGRISEUM	**Sample 1%**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0
	C. Fungicide	Grain Side	0	0	0		**C. Fungicide**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0
	Blank	Grain Side	0	0	0		**Blank**	Grain Side	0	0	0
		Flesh Side	0	0	0			Flesh Side	0	0	0

When the inbition area results were examined for *A. niger*, it could be understood that at 3 days the samples have bigger inhibition zones than the commercial and the blank samples. But the results of 7 days showed that the samples were covered by *A. niger* although the commercial and blank samples have stilll very small inhibition zones. It covered only the edges of the blank samples. But for final assessment at 14 days, it was observed that the blank samples also were covered totally by *A. niger*.

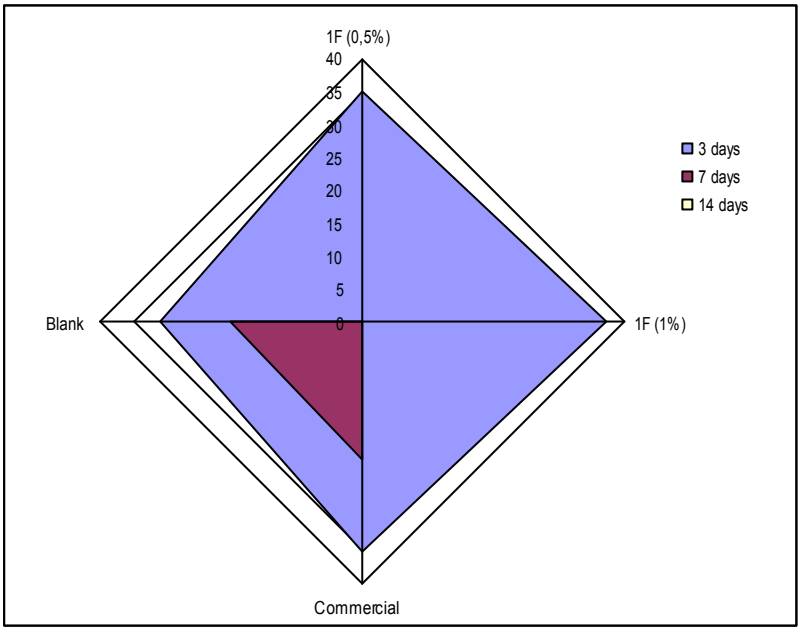


Fig. 5 Inhibition zones (mm) of *Aspergillus niger* for 3, 7 and 14 days

For *S. brevicaulis*, the results of the samples for the inhibition area were found out bigger than both commercial and blank samples for all 3 examination dates. Although the decrease of the inbition areas were seen for all the samples, benzothiazole samples gave better results especially with the one in proportion of 1%.

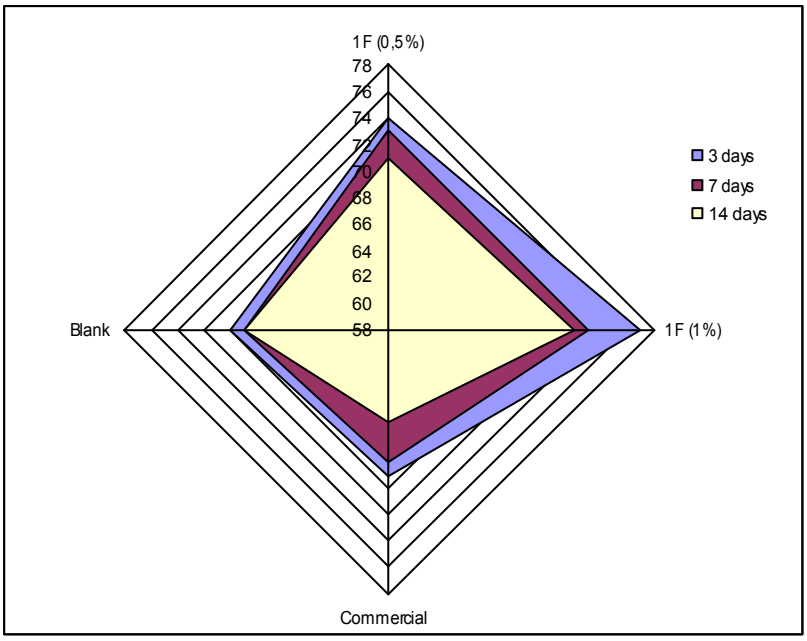


Fig. 6 Inhibition zones (mm) of *S. brevicaulis* for 3, 7 and 14 days

When the Figure 7 was examined, it could be seen that all samples gave big inhibition zones against *P. aurantiogriesum*. During the examination dates the decrease of the inhibition zones were observed but this time the samples with 2 different proportions gave the more decrease compared to blank samples. But still the sample with 1% of benzothiazole gave similiar results with the blank samples at 3 and 7 days.

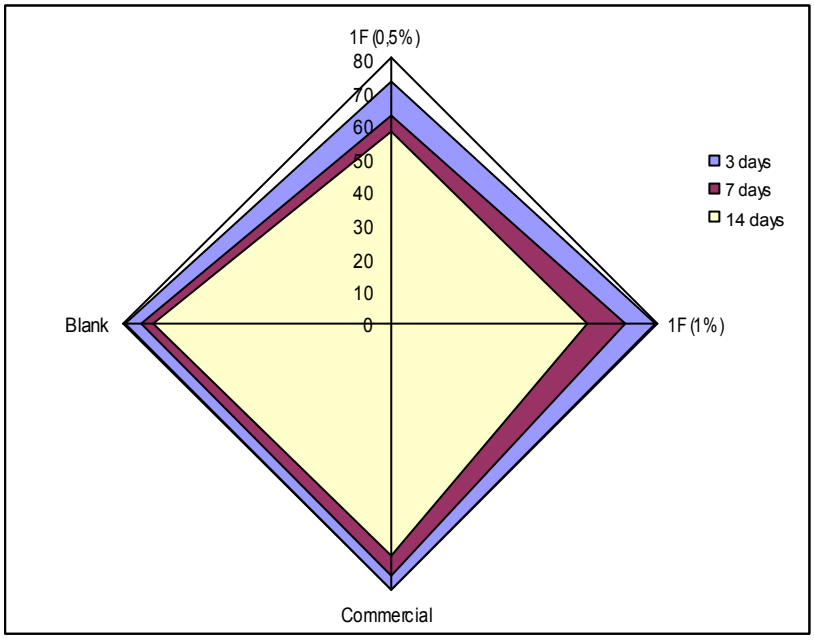


Fig. 7 Inhibition zones (mm) of *P. aurantiogriseum* for 3, 7 and 14 days

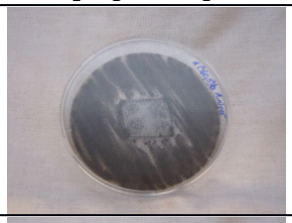
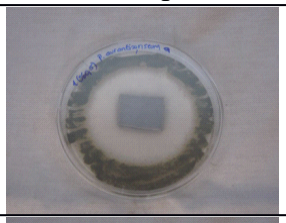
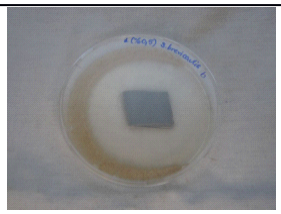
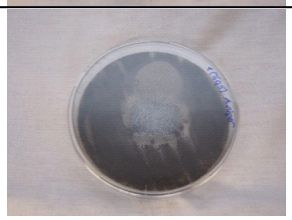
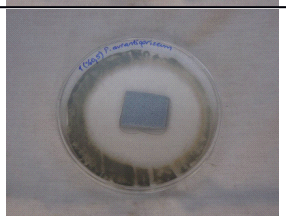
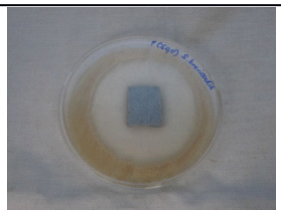
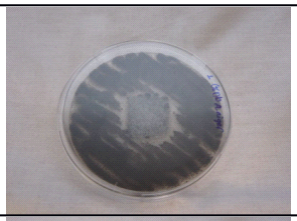
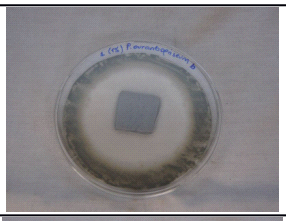
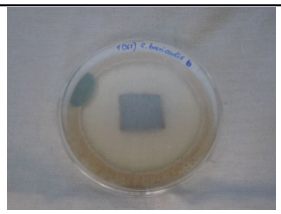
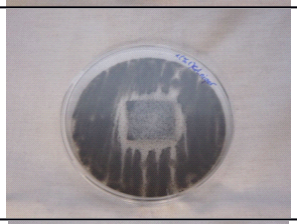
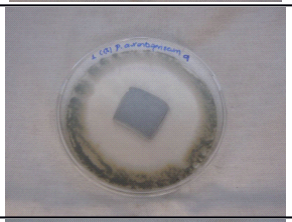
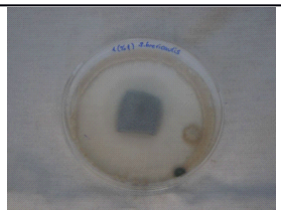
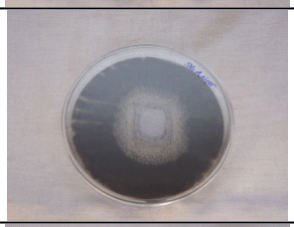
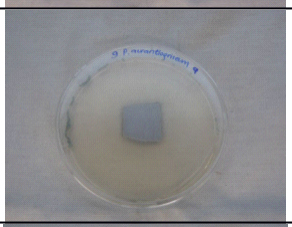
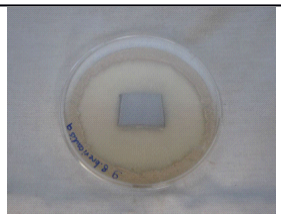
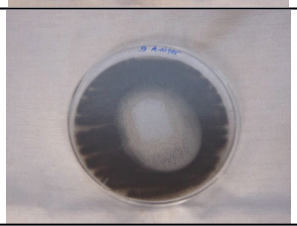
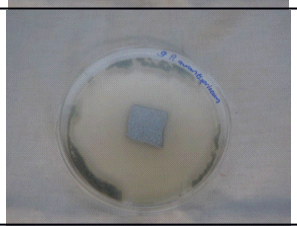
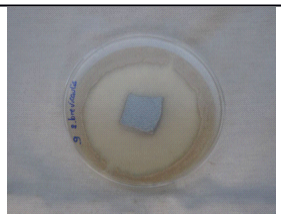
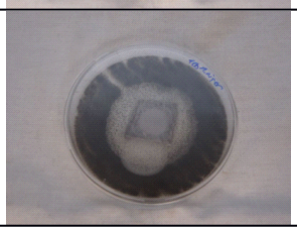
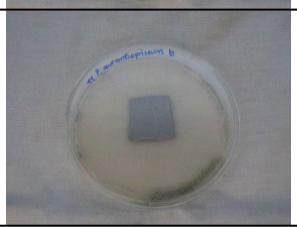
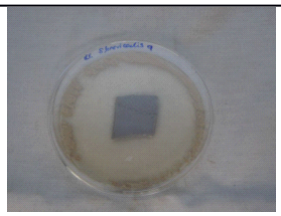
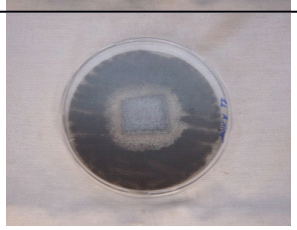
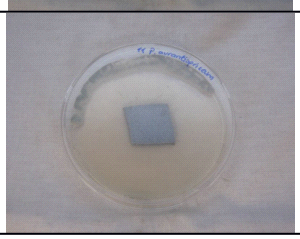
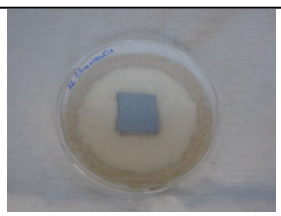
	Aspergillus niger	*P. Aurantiogriseum*	*S. Brevicaulis*
Sample Grain Side 0.5%			
Sample Flesh Side 0.5%			
Sample Grain Side 1%			
Sample Flesh Side 1%			
Commercial Fungicide Grain Side			
Commercial Fungicide Flesh Side			
Blank Grain Side			
Blank Flesh Side			

Fig. 8 Final appearance of leather samples after 14 days incubation

4 Conclusion

2-amino-6-benzothiazole substituted with methoxy in the form of sulfonic acids based fungicide was applied to bovine leathers with 2 different proportions (0.5-1%) at pickling step and their effect were investigated with 3 different fungi species.

The test organism *Aspergillus niger*, covered the benzothiazole based fungicide applied leather samples at 7 days. But for *S. brevicaulis* and *P. aurantiogriseum* fungi species, the fungicide sample has a good effectiveness for both proportions. Neither the growth of the fungi species on leather samples was observed nor were the inhibition areas nearly the edge of the leather samples. They have great inhibition areas for all 3 assessment dates and the 2 different proportions haven't make a significant difference. In the view of these findings, 0.5% of benzothiazole based fungicide is sufficient for the protection from the fungi species of *S .brevicaulis* and *P. aurantiogriseum.* Besides this, for the *A. niger* fungi specie, both of the proportions are not adequate and it has always a contamination risk after 7 days.

Although it was found out that the effect of the biocide was not satisfactory only considering the results of *A. niger*, the washing and bounding degrees of them were found better than the commercial sample and these results confirmed the aim of bounding benzothiazole fungicide to the leather. From this point of view, the different salts of benzothiazole based fungicides were decided to be synthesized for the antifungal application and this could be the solution of the unfavorable result of the *A. niger*.

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Acknowledgment

The paper was performed as part of bilateral cooperation project Romania - Turkey (*Studies on synthesis of a new benzothiazole biocide class and its application in the preservation of bovine wet-blue leather)* financed by the MECI and TUBITAK in 2008-2010.