

## THE IDENTIFICATION OF MOLD GROWN ON THE SURFACE AND EPIDERM OF THE WHEAT OBTAINED IN THE CENTRAL REGIONS OF ALBANIA

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### ABSTRACT

The purpose of this study is to create a general overview on microbiological characteristics determined on the surface and epiderm of the wheat. The wheat samples were obtained in the central regions of Albania during March- June period, 2015. Microbiological evaluation was done in 4 different media for total bacteria, yeast and mold. The total number of microorganisms grown on the surface of the wheat was determined by the method of placing the suspensions on solid medium. In the case of epiderm, the total number of microorganisms was done by treating wheat kernels with 90ml neutral detergent 0.1 %. Then it was washed four times with 90ml of sterile water. After that, it was thrown clean and sterile sand. In this study was prepared three dilutions with 2 parallels for both cases. After incubation at 26°C (for mold,) and at 30°C (for bacteria), the colonies were counted. The concentration of microorganisms in the original samples was calculated from plates with 25 - 250 colonies. The calculation of total number of microorganisms was done by assuming that each colony grew from a single cell. It was notice that the number of microorganisms grown on the surface was higher than the epiderm. It had some exceptions. A very important point of this study is the identification of mold based on colony growth, cultural and phenotypic characteristics. There was noticed mainly Ascomycete classes especially *Aspergillus spp.* In general, wheat samples which have been analyzed were within standards.

**Keywords:** Wheat, mold, surface, epiderm.

### INTRODUCTION

Wheat is probably the most common cereal available all over the world and is in even higher demand in recent years due to its abundant health benefits. Over the years, wheat has shown itself to be one of the most successful and sustainable cereals crops in the world. There are more than 150 species of filamentous fungi and yeasts on cereal grains. But again, the most important of these are the filamentous fungi or molds. The filamentous fungi that occur on cereal grains are divided into two groups, depending on when they predominate in grain in relation to available moisture in the grain.

### METHODOLOGY

The aim of this study was to create a general overview on microbiological characteristics and the identification of mold grown on the surface and epiderm of the wheat. The wheat samples were obtained in sterile condition from 5 different industrial mill companies in the central regions of Albania during March- June period, 2015. The total number of microorganisms, were determined

by the method of placing the suspensions on solid medium (Czapek dox agar, Potato dox agar, Malt extract agar, Plate count agar).

1kg of wheat was taken for each sample in order to prepare the sample average with diagonal division. There was used streptomycin 30mg in Czapek medium to inhibit bacteria growth. After incubation at 26 ° C (for mold,) and at 30 ° C (for bacteria), the colonies were counted. The concentrations of microorganisms in the original samples were calculated from plates with 25 - 250 colonies. Calculating the total number of microorganisms was done by assuming that each colony grew from a single cell. This method is conventional, because it gives approximate results, but can be judged on the density of microorganisms. The experimental work was based on:

- **The total number of microorganisms grown on the surface of the wheat**

10g wheat was taken and thrown into an Erlenmeyer with 90 ml of sterile water. There were used three dilutions and it was cultivated 2 parallels with 1ml suspension in each plate.

$$\frac{10\text{ml sample}}{10\text{ml sample} + 90\text{ml sterile water}} = \frac{10\text{ml}}{10\text{ml} + 90\text{ml}} = \frac{10\text{ml}}{100\text{ml}} = 10^{-1}$$

$$\frac{10\text{ml from 1dilution (1) } \times 10^{-1}}{10\text{ml sample} + 90\text{ml sterile water}} = \frac{1\text{ml}}{10\text{ml} + 90\text{ml}} = \frac{1\text{ml}}{100\text{ml}} = 10^{-2}$$

$$\frac{10\text{ml from 2 dilution (2) } \times 10^{-2}}{10\text{ml sample} + 90\text{ml sterile water}} = \frac{0.1\text{ml}}{10\text{ml} + 90\text{ml}} = \frac{0.1\text{ml}}{100\text{ml}} = 10^{-3}$$

- **The total number of microorganisms grown on the epiderm of the wheat kernel**

10g of wheat were treated with 90ml neutral detergent 0.1 %. The wheat was washed four times with 90 ml of sterile water. There were dumped 10 g sterile sand and 90 ml of sterile water. After shaking, it was prepared dilutions. The Petri dishes were placed in the incubator.

- **The identification of mold grown on the surface and epiderm of the wheat kernel**

The identification of mold was based on colony growth, cultural and phenotypic characteristics. They were classified in species and genus. Mold species present in wheat were determined by direct microscopic observation.

RESULTS

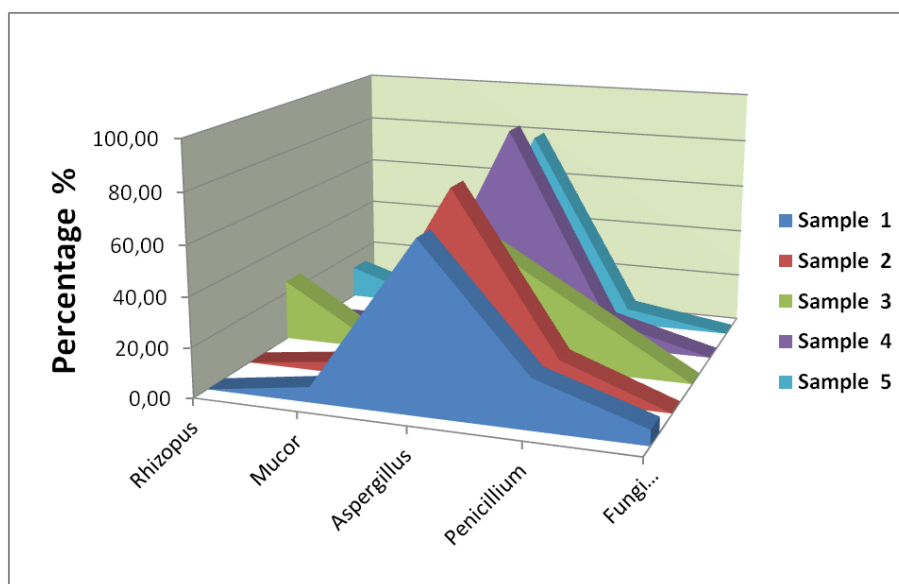
**Table 1:** The total number of microorganisms grown on the surface and the epiderm of the wheat kernel for 5 samples.

SAMPLE 1															
Dilution	Parallel	Microorganisms on the surface							Microorganisms on the epiderm						
		Bacteria			Yeast	Mold			Bacteria			Yeast	Mold		
		PCA	PDA	MA	PCA	PDA	MA	Czapek	PCA	PDA	MA	PCA	PDA	MA	Czapek
10 <sup>-1</sup>	1	too many	too many	10	0	12	0	6	too many	too many	7	0	22	0	5
	2	too many	too many	4	0	17	0	10	too many	too many	7	0	26	0	1
	Average	too many	too many	7	0	14,5	0	8	too many	too many	7	0	24	0	3
	Total	too many			70	225			too many	70	270				
10 <sup>-2</sup>	1	500	too many	2	0	2	0	8	380	too many	1	0	3	0	3
	2	480	too many	5	0	1	0	7	370	too many	5	0	4	0	4
	Average	490	too many	3,5	0	1,5	0	7,5	375	too many	3	0	3,5	0	3,5
	Total	49000			350	900			37500	300	700				
10 <sup>-3</sup>	1	447	25	0	0	0	0	2	100	23	2	0	1	0	1
	2	300	32	0	0	0	0	0	22	25	0	0	0	0	0
	Average	373,5	28,5	0	0	0	0	1	61	24	1	0	0,5	0	0,5
	Total	402000			0	1000			85000	1000	1000				
SAMPLE 2															
Dilution	Parallel	Microorganisms on the surface							Microorganisms on the epiderm						
		Bacteria			Yeast	Mold			Bacteria			Yeast	Mold		
		PCA	PDA	MA	PCA	PDA	MA	Czapek	PCA	PDA	MA	PCA	PDA	MA	Czapek
10 <sup>-1</sup>	1	too many	too many	7	0	8	0	0	50	3	5	0	0	0	0
	2	too many	too many	2	0	7	0	0	57	2	1	1	0	0	0
	Average	too many	too many	4,5	0	7,5	0	0	53,5	2,5	3	0,5	0	0	0
	Total	too many			45	75			560	30	5				
10 <sup>-2</sup>	1	55	2	0	0	0	0	0	21	10	0	0	0	0	0
	2	50	0	0	0	0	0	0	28	7	0	1	0	0	0
	Average	52,5	1	0	0	0	0	0	24,5	8,5	0	0,5	0	0	0
	Total	5350			0	0			3300	0	50				
10 <sup>-3</sup>	1	6	2	0	0	0	0	0	8	0	0	0	0	0	0
	2	9	0	0	0	0	0	0	8	0	0	0	0	0	0
	Average	7,5	1	0	0	0	0	0	8	0	0	0	0	0	0
	Total	8500			0	0			8000	0	0				

SAMPLE 3															
Dilution	Parallel	Microorganisms on the surface							Microorganisms on the epiderm						
		Bacteria		Yeast	Mold				Bacteria		Yeast	Mold			
		PCA	PDA	MA	PCA	PDA	MA	Czapek	PCA	PDA	MA	PCA	PDA	MA	Czapek
10 <sup>-1</sup>	1	too many	too many	20	0	2	0	1	17	2	14	0	0	0	0
	2	too many	too many	12	0	2	0	0	14	2	5	0	0	1	0
	Average	too many	too many	16	0	2	0	0,5	15,5	2	9,5	0	0	0,5	0
	Total	too many		160	25				175	95	5				
10 <sup>-2</sup>	1	31	12	9	0	0	0	0	2	1	5	1	0	0	0
	2	29	9	6	0	1	1	1	0	2	4	0	0	0	0
	Average	30	10,5	7,5	0	0,5	0,5	0,5	1	1,5	4,5	0,5	0	0	0
	Total	4050		750	150				250	450	50				
10 <sup>-3</sup>	1	14	1	4	0	0	0	0	2	0	3	1	0	0	0
	2	7	0	4	0	0	0	0	1	0	0	0	1	0	0
	Average	10,5	0,5	4	0	0	0	0	1,5	0	1,5	0,5	0,5	0	0
	Total	11000		4000	0				1500	1500	1000				
SAMPLE 4															
Dilution	Parallel	Microorganisms on the surface							Microorganisms on the epiderm						
		Bacteria		Yeast	Mold				Bacteria		Yeast	Mold			
		PCA	PDA	MA	PCA	PDA	MA	Czapek	PCA	PDA	MA	PCA	PDA	MA	Czapek
10 <sup>-1</sup>	1	too many	too many	8	0	1	0	1	95	10	6	0	0	0	0
	2	too many	too many	8	2	1	0	1	90	9	0	1	0	0	2
	Average	too many	too many	8	1	1	0	1	92,5	9,5	3	0,5	0	0	1
	Total	too many		80	30				1020	30	15				
10 <sup>-2</sup>	1	too many	15	3	0	0	0	0	50	4	0	1	0	0	0
	2	too many	14	0	0	1	0	1	42	2	0	0	0	0	0
	Average	too many	14,5	1,5	0	0,5	0	0,5	46	3	0	0,5	0	0	0
	Total	1450		150	100				4900	0	50				
10 <sup>-3</sup>	1	60	2	0	1	0	0	0	20	1	0	1	0	0	0
	2	58	3	0	0	0	0	0	15	1	0	0	0	0	0
	Average	59	2,5	0	0,5	0	0	0	17,5	1	0	0,5	0	0	0
	Total	61500		0	500				18500	0	500				
SAMPLE 5															
Dilution	Parallel	Microorganisms on the surface							Microorganisms on the epiderm						
		Bacteria		Yeast	Mold				Bacteria		Yeast	Mold			
		PCA	PDA	MA	PCA	PDA	MA	Czapek	PCA	PDA	MA	PCA	PDA	MA	Czapek
10 <sup>-1</sup>	1	too many	too many	4	1	0	0	3	too many	5	3	0	0	0	0
	2	too many	too many	0	0	1	1	4	too many	5	0	1	0	0	2
	Average	too many	too many	2	0,5	0,5	0,5	3,5	too many	5	1,5	0,5	0	0	1
	Total	too many		20	50				50	15	15				
10 <sup>-2</sup>	1	15	10	0	0	0	2	0	20	4	0	0	0	0	0
	2	8	8	0	0	1	1	0	15	2	0	0	0	0	0
	Average	11,5	9	0	0	0,5	1,5	0	17,5	3	0	0	0	0	0
	Total	2050		0	200				2030	0	0				
10 <sup>-3</sup>	1	2	2	0	0	0	0	0	9	0	0	0	0	0	0
	2	1	2	0	0	0	1	0	7	0	0	1	0	0	0
	Average	1,5	2	0	0	0	0,5	0	8	0	0	0,5	0	0	0
	Total	3500		0	500				8000	0	500				

**Table 2:** The identification and classification of mold grown on the surface of the wheat.

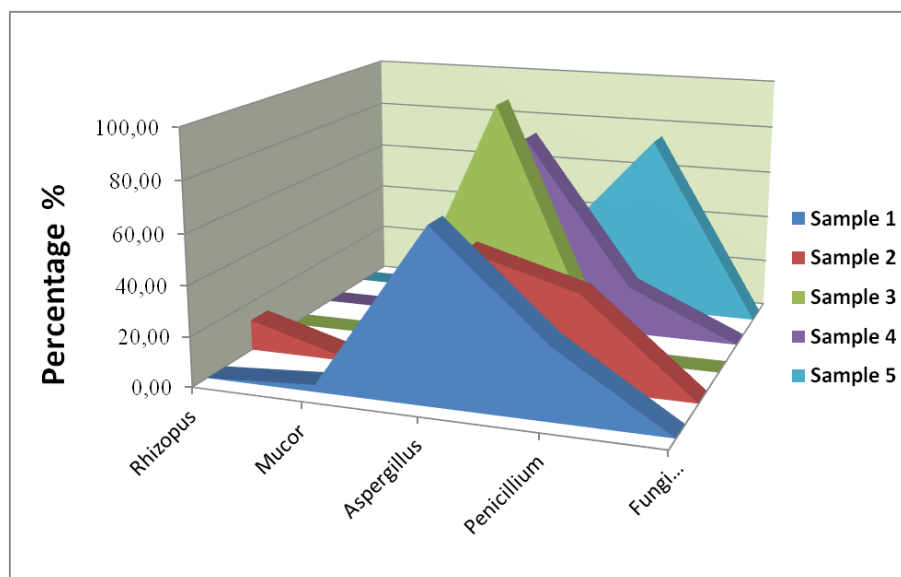
Class	Phycomycetes		Ascomycetes				Fungi imperfecti		
	Rhizopus	Mucor	Aspergillus		Penicillium				
Sample 1		<i>Mucor</i>	4	<i>A.candidus</i>	35	<i>Penicillium spp.</i>	12	<i>Trichothecium roseum</i>	2
				<i>A.flavus</i>	7	<i>P.rubrum</i>	1	<i>Helminthosporium spp.</i>	2
				<i>A.terreus</i>	2				
<b>Total</b>	<b>0</b>	<b>4</b>	<b>44</b>	<b>13</b>	<b>4</b>	<b>13</b>	<b>4</b>		
Sample 2		<i>Mucor</i>	1	<i>A.candidus</i>	8	<i>Penicillium spp.</i>	3		
				<i>A.flavus</i>	7				
<b>Total</b>	<b>0</b>	<b>1</b>	<b>15</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>0</b>		
Sample 3	<i>R.nigricans</i>	2		<i>A.candidus</i>	2	<i>Penicillium spp.</i>	1		
				<i>A.flavus</i>	2	<i>P.islandicum</i>	1		
<b>Total</b>	<b>2</b>	<b>0</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>0</b>		
Sample 4				<i>A.candidus</i>	4	<i>Penicillium spp.</i>	1		
				<i>A.flavus</i>	3				
				<i>A.versicolor</i>	1				
<b>Total</b>	<b>0</b>	<b>0</b>	<b>8</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>		
Sample 5	<i>R.nigricans</i>	2		<i>A.candidus</i>	9	<i>Penicillium spp.</i>	1		
				<i>A.flavus</i>	1				
				<i>A.terreus</i>	1				
				<i>A.niger</i>	1				
<b>Total</b>	<b>2</b>	<b>0</b>	<b>12</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>		



**Graph 1:** Distribution, in percentage %, of mold grown on the surface of the wheat.

**Table 3:** The identification and classification of mold grown on the epiderm of the wheat

Class	Phycomycetes		Ascomycetes		Fungi imperfecti	
Genus	Rhizopus	Mucor	Aspergillus	Penicillium		
Sample 1		<i>Mucor</i> 2	<i>A.candidus</i> 44	<i>Penicillium spp.</i> 20		
			<i>A.flavus</i> 2			
			<i>A.terreus</i> 2			
<b>Total</b>	<b>0</b>	<b>2</b>	<b>48</b>	<b>20</b>	<b>0</b>	
Sample 2	<i>R.nigricans</i> 1		<i>A.candidus</i> 2	<i>Penicillium spp.</i> 3		
			<i>A.flavus</i> 2			
<b>Total</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>3</b>	<b>0</b>	
Sample 3			<i>A.candidus</i> 3			
			<i>A.flavus</i> 1			
<b>Total</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>0</b>	
Sample 4			<i>A.candidus</i> 2	<i>Penicillium spp.</i> 1		
			<i>A.flavus</i> 1			
			<i>A.versicolor</i> 1			
<b>Total</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>1</b>	<b>0</b>	
Sample 5			<i>A.flavus</i> 1	<i>Penicillium spp.</i> 1		
				<i>P.islandicum</i> 2		
<b>Total</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>0</b>	

**Graph 2:** Distribution, in percentage %, of mold grown on the epiderm of the wheat.

## DISCUSSION

As mentioned in the methodology, there were analyzes 5 samples. There were determined the total number of microorganisms on the surface and the epiderm of the wheat. Results of the definitions are presented in the following table: Table 1. In all cultivation cases, in first dilution, was distinguished a high microbiological weight. So the calculation of total number of bacteria, yeast and mold was done from plates with 25 - 250 colonies.

The results were observed that the total number of bacteria grown on the surface were some times higher than the epiderm. There was one exception for sample 4, wherein the total number of bacteria was three times higher in epiderm than the surface. There was mainly *Pseudomonas herbicola* species which are characteristics for young wheat. The total number of yeast were little higher on the surface than the epiderm. The total number of mold was two times higher on the surface than the epiderm. There was one exception for sample 1, wherein the total number of mold was little higher on the epiderm than the surface.

A very important point of this study was the identification of mold which was grown in all media. The identification of mold was based on colony growth, cultural and phenotypic characteristics. Some of them were determined by direct microscopic observation. By identification of mold and classification in species and genus there was noticed mainly Ascomycete class especially *Aspergillus spp.* and *Penicillium spp.*. *Aspergillus spp.* especially *A.candidus* and *A.flavus* are characteristic of stored grain. This shows that wheat used in industrial mill companies are not young, but are stored wheat for a while.

In general wheat samples which have been analyzed were within standards. The results are presented in, Table 2, Table 3, Graph 1 and Graph 2. The sample with the highest number of mold, especially *Aspergillus spp.* was sample 1.

## CONCLUSIONS

Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The sources of microbial contamination of cereals are many, but all are traceable to the environment in which grains are grown, handled, and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment. From all the experimental work, we may recommend that the industrial mill companies should pay attention to preparation for milling processes, such as: cleaning, sorting, conditioning of the wheat because wheat is the basic food in our life.

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