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Microfungus Flora of Rice (*Oryza sativa* L.) and Wheat (*Triticum durum* L.) Grains from Corum Region (Turkey)

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Abstract
In this study, it was aimed to isolate and define the microfungi flora on rice (*Oryza sativa* L. var. Osmancik – 97) and wheat (*Triticum durum* L.) cultivated in Corum province (Turkey). After macroscopic and microscopic criteria, microfungi colonies were defined at the species level. Along with the study, in rice grains, *Aspergillus clavatus, Aspergillus niger, Cladosporium orchidis, Penicillium commune, Penicillium crustosum, Penicillium pallidum,* and *Penicillium brevicompactum* were recorded whereas In wheat grains; *Alternaria alternata, Curvularia lunata, Curvularia sorghina, Fusarium poae, Microsporon audouinii, Penicillium albicans, Penicillium chrysogenum, Penicillium funiculosum, Penicillium gladioli,* and *Ulocladium botrytis* were defined and reported fot wheat samples.

Keywords: Rice, Wheat, Microfungi, Corum, Turkey.

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1. Introduction

Plant seeds are considered highly effective means of transporting plant pathogens over long distances. There are many studies in the agricultural literature on the international spread of plant diseases as a result of importing seeds contaminated with pathogens. Seed borne diseases have been reported to significantly affect plant growth and productivity (Agarwal and Srivastava, 1981; Dhakar and Jat, 2017). Therefore, agricultural crops and products are of the essential issues for the regions importing the crops and other products.

Cereal products can be contaminated through two different ways, which are sorted as field or plant pathogens contamination (*Fusarium* spp.) as well as with storage or saporphytic (*Aspergillus* and *Penicillium* spp.) (Glenn, 2007; Mohamed et al., 2017).

Based on the previous previous reports, wheat and rice grains can be source of mainly *Aspergillus, Fusarium Penicillium* which are responsible for common mycotoxin contaminants (Al-Hazmi, 2010; Bensassi et al., 2011; Lutfullah and Hussain, 2012; Mohamed and Shereen, 2017).

Herewith, rice (*Oryza sativa* L.var. Osmancık-97) and wheat (*Triticum durum* L.) grains collected in Çorum regions were monitored for their microfungi flora.

2. Materials and Methods

Preparation of samples

Rice (*Oryza sativa* L. var. Osmancık-97) and wheat (*Triticum durum* L.) were collected in Çorum regions. Wheat and rice grains (2008 crops) were subjected to humidification in vitro for one week. One gram of samples were taken from the moistened wheat and rice samples at the end of one week. For homogenized rice and wheat samples, a dilution of 1/3 (for each sample) up to 10^{-3} was made (Xie et al., 2007).

Cultivation medium and incubation

Streptomycine-Rose Bengal Agar (SRBA) (Pitt, 1985), Potato Dextrose Agar (PDA) (Watanabe, 2002), Czapek Agar (CA), (Larone, 1993), Malt Extrakt Agar (MEA) (Samson et al., 2000) media were used.

The macroscopic and microscopic criteria of the fungi colonies that developed at the end of the incubation period (7-14 days at 25 ºC) were examined and identified (Harrigan and McCance, 1976; İlhan and Asan, 2001; Karabulut ve Değirmencioğlu, 2002; Mohamed and Shereen, 2017).

Identification of microfungus

Identification of microfungi for *Aspergillus*; Samson et al. (2000), Klich (2002) for *Penicillium*; Pitt (1985), Larone (1993), Samson et al. (2000), and Watanabe (2002) were used whereas other species were defined with Hasenekoglu (1991) and Domsch et al. (1995).

3. Results and Discussion

In this study, microfungi flora of rice (*Oryza sativa* L. var. Osmancık-97) and wheat (*Triticum durum* L.) grown in Çorum and in the region were investigated in vitro. *Alternaria alternata, Curvularia lunata, Curvularia sorghina, Fusarium poae, Microsporon audouinii, Penicillium albicans, Penicillium chrysogenum, Penicillium funiculosum, Penicillium gladioli, Ulocladium botrytis, Aspergillus clavatus, Aspergillus niger, Cladosporium orchidis, Penicillium commune, Penicillium crustosum, Penicillium pallidum,* and *Penicillium brevicompactum* species were identified (Table 1).

Table 1. Species identified in rice (Oryza sativa L.) and Wheat (Triticum durum L.) seeds

Species name	Found sample	Fungi (%)	Reference
***Alternaria* Nees ex Fr.**		**6**	
Alternaria alternata (Fr.) Keissl.	Wheat		Hasenekoğlu (1991)
***Aspergillus* Mich. Ex Fr.**		**11.75**	
Aspergillus clavatus Desm.	Rice		Samson et al (2000), Klıch (2002)
Aspergillus niger van Tieghem.	Rice		Samson et al (2000), Klıch (2002)
Cladosporium Link**:** Fries		**6**	
Cladosporium orchidis E.A. & M.B. Ellis.	Rice		Hasenekoğlu (1991).
Curvularia Boedijn**.**		**11.75**	
Curvularia lunatus (Wakker) Boedijn	Wheat		Domsch et al (1980)
Curvularia soghina R.G. Shivas & Sivan.	Wheat		Hasenekoğlu (1991)
***Fusarium* Link ex Fr.**		**6**	
Fusarium poae (Peck) Wollenw.	Wheat		Hasenekoğlu (1991)
***Microsporon* Gruby.**		**6**	
Microsporon audouinii Gruby.	Wheat		Hasenekoğlu (1991)
***Penicillium* Link ex Gray.**		**47**	
Penicillium albicans Bainier.	Wheat		Samson et al (2000), Klıch (2002)
Penicillium chrysogenum Thom.	Wheat		Samson et al (2000), Klıch (2002)
Penicillium commune Thom.	Rice		Samson et al (2000), Klıch (2002)
Penicillium crustosum Thom *(farinosum* Nov.)	Rice		Samson et al (2000), Klıch (2002)
Penicillium funiculosum Thom.	Wheat		Samson et al (2000), Klıch (2002)
Penicillium gladioli L. McCulloch & Thom.	Wheat		Samson et al (2000), Klıch (2002)
Penicillium pallidum G.Sm(Syn:*Geosmithia putterillii* Thom)	Rice		Samson et al (2000), Klıch (2002)
Penicillium brevicompactum Dierckx.*(stoloniferum* Thom.)	Rice		Samson et al (2000), Klıch (2002)
***Ulocladium* Preuss**		**6**	
Ulocladium botrytis Preuss	Wheat		Hasenekoğlu (1991)

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Molds in food can be toxic, especially on wheat and rice, which are commonly consumed cereals. Table 1 shows the % of fungi defined in the grains of *Oryza sativa* L. and *Triticum durum* L. collected in Çorum regions. During the study, *Triticum durum* L. grains; *Penicillium* (23.5%), *Curvularia* (11.75%), *Fusarium* (6%), *Ulocladium* (6%), *Alternaria* (6%), *Microsporon* (6%) and *Oryza sativa* L. seeds; *Penicillium* (23.5%), *Aspergillus* (11.75%), *Cladosporium* (6%) genus microfungus species were identified (Table 1).The number of *Penicillium* species identified from *Triticum durum* and *Oryza sativa* seeds is the highest compared to other genus and is found in 23.5% of each sample. The total number of *Penicilium* species is higher than the number of other species. The total number of microfungi species of *Triticum durum* was 10 (62.5%), whereas it was 7 (37.5%) in *Oryza sativa*. In addition, 3 (33.3%) were identified in *Oryza sativa* and 6 (66.7%) were identified in *Triticum durum*. In other words, the *Triticum durum* situation is also richer in terms of microfungus genus and species diversity than *Oryza sativa* (Figure 1).
Penicillium species on rice due to the synthesis of secondary metabolite products, consumption of these products have been reported to affect human health in many studies (Ficher et al., 1992; Suttajit, 2000; Signaboubo et al., 2016). Rice (*Oryza sativa* L.) grown in Çorum and its vicinity with nutritional and economic importance can be expected to produce mycotoxin by identifying *P. commune*, *P. crustosum*, *P. pallidum*, *P. brevicompactum* species.

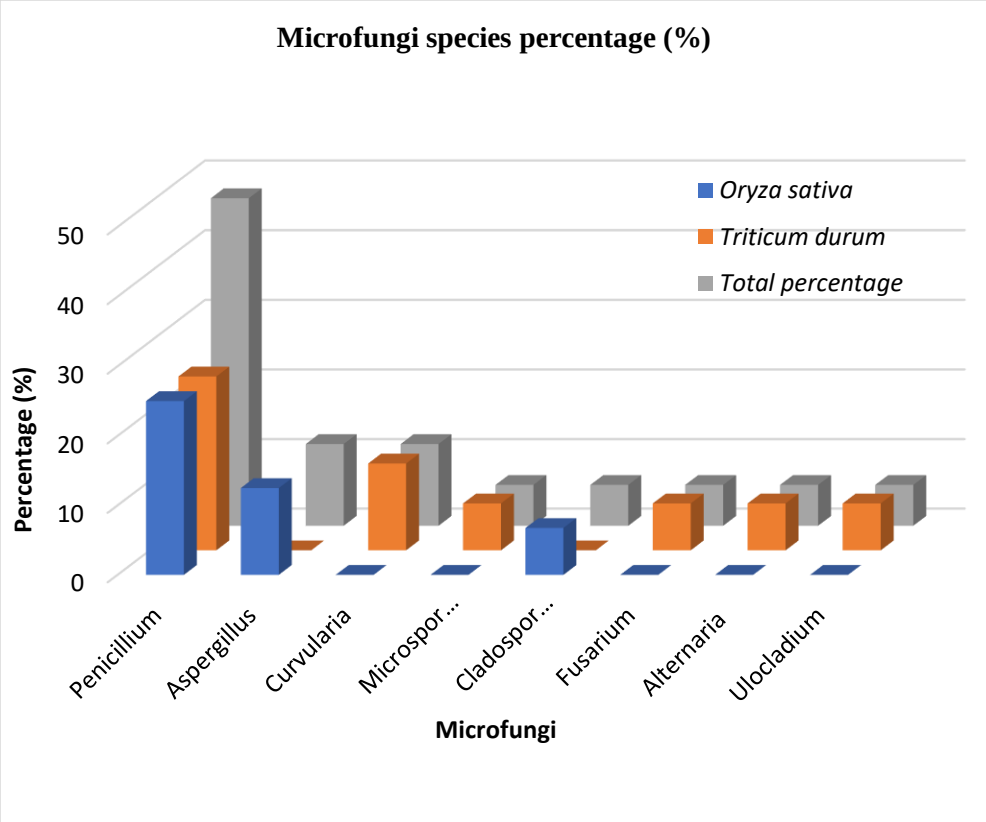

Figure 1. Percentages of fungus species in rice and wheat samples

Colonies of different dilutions (10^{-2}, 10^{-3}) can be counted on *Triticum durum* L. in PDA medium; *Oryza sativa* L. samples showed excessive (∞) growth at the same dilution rates. Significant decreases were observed in the number of wheat and rice in SRBA cultivated at the same dilutions (Table 2).

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Table 2. Microfungi propagated in the media and the number of species identified

Culture Media	**PDA**						**SRBA**	
Dilution	W*(10^{-1})	W*(10^{-2})	W*(10^{-3})	R**(10^{-1})	R**(10^{-2})	R**(10^{-3})	W*(10^{-3})	R**(10^{-3})
Number of colonies (cfu/g)	∞	278	256	∞	∞	∞	46	184
Number of species identified (pcs)		8			4		2	3

* B: Wheat **R: Rice

Likewise, *Oryza sativa* L. samples were grown 4 times more in Rose bengal medium than *Triticum durum* L.. Therefore, *Oryza sativa* L. taxa is weaker than *Triticum durum* L. and *Oryza sativa* L. can be recycled faster in nature (Table 2).
In a study conducted in Italy, aflatoxin-producing species of *Aspergillus* were found to be above the legal levels of mycotoxin in contaminated feed and in milk of animals fed with it (Giorni et al., 2007). For this reason, *Aspergillus* species should be examined for aflatoxin.
It can be said that microfungi can be formed in almost any environment under suitable humidity and temperature conditions (Adeyeye, 2016; Bradford et al., 2018), and it has suitable climatic conditions for the growth of microfungus and toxin production on cereals especially in rice due to its geographical location.
According to Abbott (2002); It has been reported to cause Alimentary Toxic Aleukia (ATA) by ingestion of Fusarium poae infected grains. The same species found in our study showed parallelism and could be investigated more widely to produce ATA (mycotoxin).
The findings of the study are in parallel with the previous studies because they are in interaction with the air (İmalı et al., 2008). Therefore, *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp. were isolated from rice (*Oryza sativa* L. var.) in wheat (*Triticum durum* L.); *Curvularia* spp., *Fusarium* sp., *Uloclodium* sp., *Alternaria* sp. genus have been identified, some of which are thought to be allergens.
Frisvad et al., (2007) compared to microfungi *Aspergillus niger* produced by Fumonosin B2 mycotoxin has a carcinogenic effect and also has similarities with *Fusarium* species and may be dangerous in terms of food safety due to the presence of many foodstuffs around us. In our study, *Aspergillus niger* was identified in rice grains and it is hoped to shed light on the following studies in terms of mycotoxic properties of the species.
Over the last 10 years, European wheat products have been increasingly influenced by Fusarium head blight (FHB) infection and high Deoxynivalenol (DON) concentrations in grains. It is argued that this is mainly caused by preventive agricultural practices and cultivation of crops such as wheat and corn simultaneously expanding (Koch, 2006). Turkey's is an important granaries to be due to the cultivation of agricultural land and improvement of the wheat and rice are also taking measures to avoid causing the same of the designated microfungi infection, also it aims to pioneer in the study.
In this study; microfungi have been identified on the level of species, the diagnosis and methods to be used in the prevention of mycotoxins, the procedures to be carried out in the way of protection from these and future studies are expected to constitute a source.

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