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RESEARCH ARTICLE

Mycoflora of Stored Wheat in Bafra District of Samsun Province

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ABSTRACT

In this study, the fungal flora in wheat grain samples taken six months after harvest from warehouses in 13 villages of Bafra district of Samsun province was investigated. A total of 600 seeds were processed isolates of endophytic, saprophytic, or pathogenic fungi recovered were identified as 15 fungal genera. *Alternaria alternata, Alternaria* spp., *Chaetomium* spp., *Phoma* spp., *Epicoccum nigrum* were the fungi that showed the highest colonization frequency in analyzed grain. Fungi such as *Penicillium, Aspergillus, Fusarium graminearum, F. poae*, which are known to produce mycotoxins, were among the isolated fungi. *Fusarium graminearum, F. poae* and *Bipolaris sorokiniana* are among the important pathogens of wheat. The other microorganisms were present at intermediate or low values. On the other hand, fungi such as *Chaetomium, Epicoccum nigrum, Torula* species were isolated as antagonist organisms. *Stemphylum, Ulocladium, Cladosporium, Popularia, Nigrospora oryzae*, which are thought to be saprophytes or endophytes, were also isolated. Some are also known as weak pathogens. On average, 31.5% of the seeds examined had one or more fungal infections, while 68.5% had no fungal infections.

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1. Introduction

Wheat is one of the most cultivated and consumed agricultural products by mankind since the day it was cultivated. It provides about 20% of the daily caloric requirement on average and about 21% of the daily protein intake in the human diet (Shiferaw et al., 2013). Wheat grains are also widely used in the flour and bakery sector as well as in the fermentation industry to produce beer, alcohol, vodka and biodiesel. Most of the products in the cereal group are stored in various ways for future use after harvest. The preferred general storage method for most of the other cereals, particularly wheat, is storage in silos or stacks in warehouses (Ertugay, 2010; Muir, 1980; Olgun, 2011). In these storage methods, the moisture content of the grain to be stored and the storage temperature are two important factors affecting the storage time of the grains (Muir, 1980). The moisture content of storage of 14% or less for wheat, ensures safe and long-lasting preservation (Wallace et al., 1983). Wolter (1986) reported that the positive effect of temperature on the flour quality extent of within 10˚C to 30˚C in stored wheat. Generally, storage temperature is kept at 15˚C and below to reduce metabolic activity and variability in stored grains (Timm et al., 2020; Wallace et al., 1983). Wheat grains can be infected by many microorganisms in the field condition or at post-harvest, which has a significant negative impact on food safety and product quality. Species belonging to fungal

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genera such as *Fusarium*, *Penicillium*, *Alternaria* and *Aspergillus* cause spoilage in stored wheat and the formation of mycotoxins that adversely affect human and animal health (Magan et al., 2011; Placinta et al., 1999; Solanki et al., 2021).

In this study, it was aimed to determine the fungal flora in stored wheat grain samples collected from different villages in Bafra district of Samsun province and to identify the fungal genera and species based on their morphological characteristics.

2. Materials and Methods

This study was carried out in 13 different villages of Bafra district (41°38′23″ North; 35°59′7″ East, Elevation = 20 m) of Samsun province, where wheat is cultivated and collected from 21 different wheat stores in 2017. The warehouses were selected as closed warehouses belonging to farmers or closed areas where bagged products were located. Sampling was done from the products stored within 6 months after harvest.

These villages in Bafra district of Samsun province include Yakıntaş, Tütüncüler, Evrenuşağı, Karıncak, Koşuköyü, Yeşilyazı, Kuşcular, Kaygusuz, Emenli, Harız, Azay, Çataltepe, Gökçeağaç villages. In the process of determining the villages where the study was carried out, it was important that the villages were in different locations and topographical features from each other. Totally, twenty samples were collected from stores in located these villages. The collected wheat samples were kept in sealed paper envelopes and brought to the laboratory.

Each collected sample batch was divided into two parts and the part not to be used for isolation was kept for further studies. From the remaining sample batch, thirty wheat grains were randomly selected. For surface disinfection, these grains were soaked in 1% NaOCl for 3 minutes, washed with distilled water and dried on blotting paper. After drying, the grains were placed on Potato Dextrose Agar (PDA) medium (39 g per liter distilled water with sterilized for 15 minutes at 121˚C and added Streptomycin sulfate in 0.1 g L^{-1} and Oxytetracycline dehydrate 0.05 g L^{-1}) in 3 replicates with 10 seeds in each petri dish. The petri dishes were then sealed with parafilm and left to incubate for 5-8 days in cabinets with a temperature of 24±1˚C and Black Light with 12 hours of light and 12 hours of darkness (Booth, 1977; Burgess et al., 1994; Nelson et al., 1983).

At the end of approximately 1 week, cultures with mycelial growth were examined under a light microscope. The seeds in the petri dishes were evaluated one by one and the fungi were identified, and the number of affected seeds was recorded. Barnett and Hunter (1998) were used for identification some fungi that did not develop spores and resembling *Fusarium* sp. transferred on CLA (Carnation Leaf Agar) medium. All identified fungi and other identified fungi on carnation leaf agar (CLA) media were transferred to ½ PDA media and stored in the refrigerator at 4˚C for a certain period.

The SPSS v21 statistical packages (IMB, Statistic, OMU Licensed for online users) were used analysis of differences between the variances by One-Way ANOVA. The variance homogeneity was analysis Levene Test (Levene, 1960) and means were grouped by Duncan multiple range test (Duncan, 1955).

3. Results

In this study, 600 wheat seeds from 21 different storage samples were examined to investigate the fungal flora in wheat grains stored for 6 months after harvest. As a result, 68.5% (411) of the seeds examined did not show any fungal growth, while 38.5% (189) were found to be infected with at least one fungal genus or species (Figure 1). A total of 192 isolates belonging fifteen fungal genera were obtained from 189 wheat seeds in which fungal growth was observed.

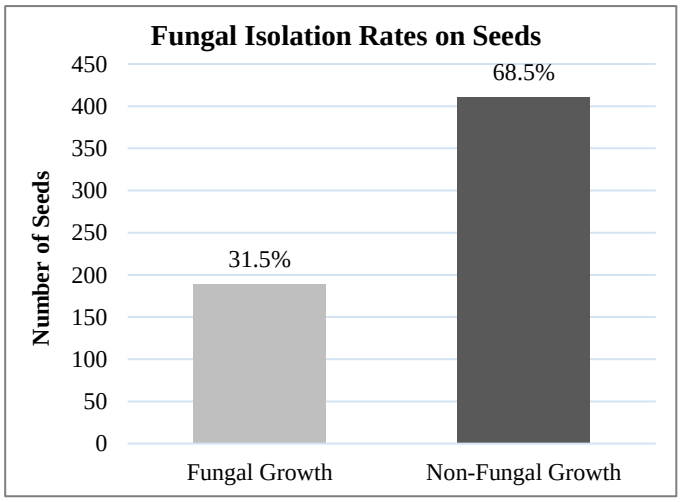

Figure 1. The percentage and number of fungal growth/non-growth on stored wheat seeds.

Fungal isolates obtained from the seeds on PDA were varied to base on their morphological characteristics and they were identified under the light microcopy. *Alternaria alternata* was the most dominant species with 41.5% among the isolated fungi from the seeds and together with other *Alternaria* isolates was the most common fungal genera with 60.4% in this study (Table 1). The morphology of *Alternaria* is typical and its conidia are dark colored, typically elliptical or spherical in shape, with both transverse and longitudinal compartments (Figure 2A and B). Following these, *Chaetomium* with 14.5% and *Phoma* with 6.7% were observed second and third most common fungal genera, respectively. The genus *Chaetomium* is generally characterized by rounded, ovoid, or obovate ostiolate ascomata covered with characteristic hairs (Figure 2C). *Epicoccum*, *Cladosporum* and *Penicillium* were among the other common fungi with 3.2%, 3% and 2.6%, respectively (Figure 2D and E). *Fusarium* was less common at 2.1%, but three different species

had been identified as *F. graminearum*, *F. tabacinum* and *F. poae* (Figure 2F, G, H). *Bipolaris*, *Ulocladium* and *Septonema* were represented by two isolates for each genus, while *Aspergillus*, *Stemphylium*, *Nigrospora*, *Torula*, *Papularia* were rarely identified as only one isolate for each (Figure 2).

Table 1. The fungal genera/species and isolation rate of stored wheat seeds.

Fungal Genera & Species	No. Isolates	Isolation Rate* (%)	Std. Deviation	Groups**
Alternaria spp.				
Alternaria alternata	*79*	41.46	±3.40	a
Alternaria others	*37*	18.96	±5.32	ab
Fusarium spp.				
Fusarium graminearum	*2*	0.96	±0.86	e
Fusarium tabacinum	*1*	0.50	±0.86	e
Fusarium poae	*1*	0.63	±1.09	e
Bipolaris spp.				
Bipolaris spicifera	*1*	0.63	±1.09	e
Bipolaris sorokiniana	*1*	0.63	±1.09	e
Phoma spp.	*13*	6.66	±1.42	d
Aspergillus sp.	*1*	0.50	±0.86	e
Penicillium spp.	*5*	2.63	±3.06	de
Epicoccum nigrum	*6*	3.23	±1.62	de
Chaetomium spp.	*29*	14.50	±6.06	c
Cladosporum spp.	*6*	3.03	±1.15	de
Septonema spp.	*2*	1.13	±1.00	e
Stemphylium sp.	*1*	0.63	±1.09	e
Ulocladium spp.	*3*	1.73	±1.92	e
Torula sp.	*1*	0.63	±1.09	e
Nigrospora oryzae	*1*	0.63	±1.09	e
Papularia sp.	*1*	0.50	±0.86	e
Sterile fungus	*1*	0.50	±0.86	e

* There is a significant difference among the variances (F=54.74, df=19, $p<0.01$).
**Duncan multiple range test.

4. Discussion

When the seeds were examined randomly by eye although a few seeds were chalky, embryos were blackened and spindly, 95% of the seeds appeared healthy. It is thought that most of the fungi obtained from these seeds may be endophytes. Researchers have shown that endophytes have an effect on plant growth and that phytohormones, such as indole-3-acetic acid, cytokinin, and other plant growth regulators play a role in increasing plant growth (Tan & Zou, 2001). Some researchers have also reported that endophytes contribute to the uptake of nutrients such as nitrogen and phosphorus by the host (Malinowski & Belesky, 1999; Reis et al., 2000). Gibberellins also play an important role in plant development. However, 12 fungal species have been found to produce gibberellins so far (Kawaide, 2006; MacMillan et al., 2005; Vandenbussche et al., 2007). In one study, the gibberellin production capacity of 19 endophytic fungal isolates was determined in Waito-C paddy cultivar and their effect on shoot growth was investigated, and more plant height growth was recorded in cucumber plants compared to the control (Hamayun et al., 2010).

On the other hand, it is known that isolates of *Torula*, *Ulocladium*, *N. oryzae*, *E. nigrum*, *Penicillium* and *Aspergillus* species are saprophytic (Barnet & Hunter, 1998). However, it should be taken into consideration that *Aspergillus* and *Penicillium* species, for example, produce mycotoxins (Prasher et al., 2024). It is known that about 250 of the fungal species whose existence has been revealed to date produce mycotoxins and about 20 of them cause poisoning in humans and animals (Erdem & Özen, 1990).

In a study on mycotoxin formation in cereals, it was stated that there is a very suitable environment for mycotoxin formation in cereals. The probability of formation of aflatoxin B1 and other aflatoxins in stored and carbohydrate-rich foodstuffs such as wheat and flour are very high (Evren, 1999). Aflatoxins are known to have toxigenic, mutagenic, teratogenic and carcinogenic effects for humans and animals (Ünlütürk & Turantaş, 1998). Aflatoxin formed by *Aspergillus* species and

ochratoxin A formed by *Penicillium* species are the leading mycotoxins that cause significant health problems. The mycotoxins mainly grow on the food before or after harvesting and during storage. Most mycotoxins are chemically stable, and they survive food processing.

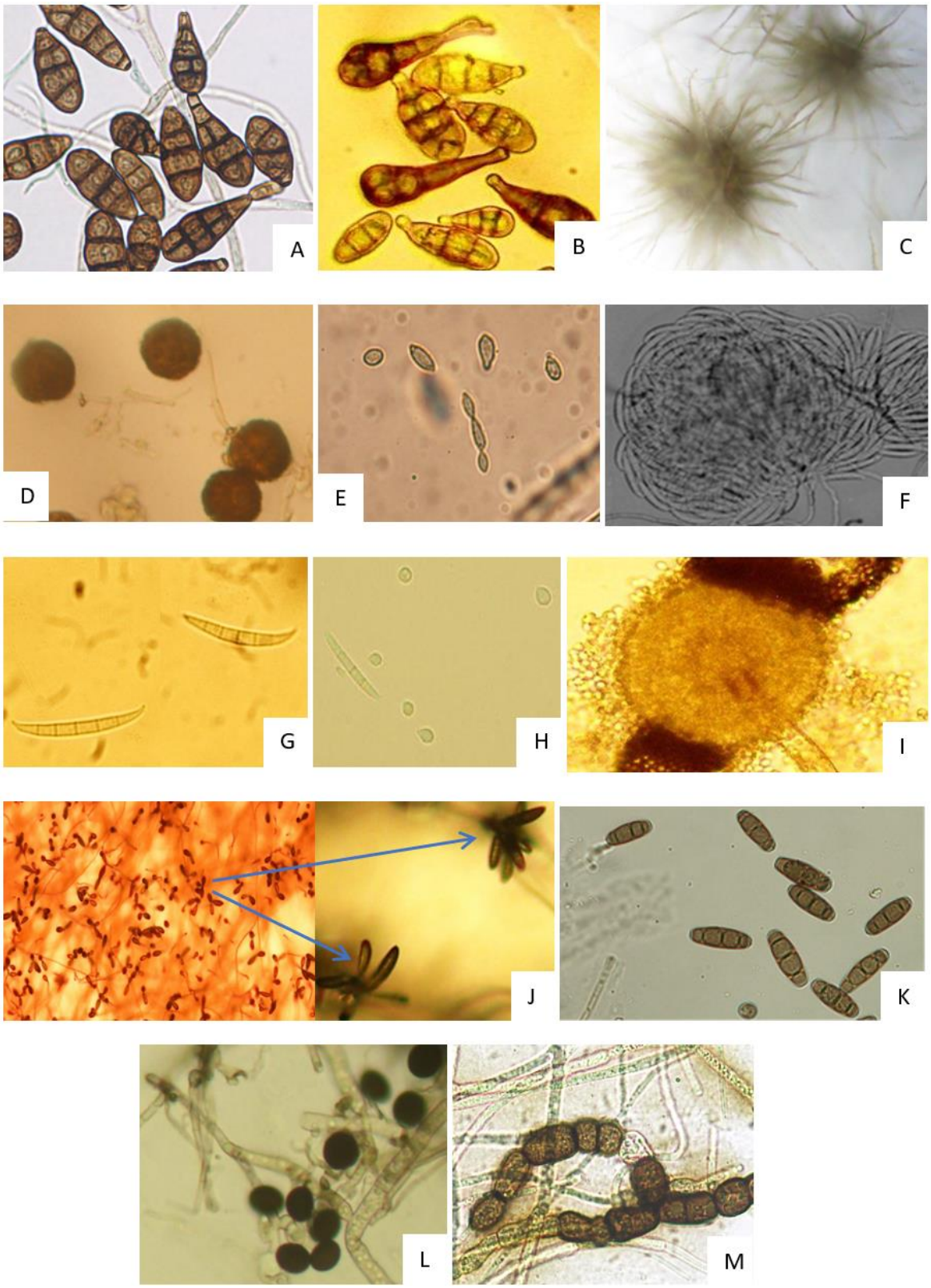

Figure 2. The microscopic images of fungal genus/species: A=*Alternaria alternata*, B= *Alternaria* sp., C= *Chaetomium* spp., D= *Epicoccum nigrum*, E= *Cladosporum* sp., F= *Fusarium graminearum* sporodochium, G= *F. graminearum* macroconidium, H= *Fusarium poae*, I= *Aspergillus* sp., J= *Bipolaris sorokiniana* conidia, K= *Bipolaris spicifera* conidia, L= *Nigropora oryzae*, M= *Torula* sp.

When the results are analyzed, it is seen that *Alternaria* spp. is the most common genus in wheat seeds and *A. alternata* is the most common species. In some other studies, it was determined that *A. alternata* was the most common species in the leaves, stems, ears and grains of wheat plants (Larran et al., 2002, 2007). The most frequently detected Alternaria toxins, which have significant toxicity, are alternariol (AOH), alternariol monomethyl ether (AME), altertoxins (ATXs; I, II and III), altenuene (ALT), tenuazonic acid (TEA), tentoxin (TEN) and *A. alternata* f. sp. *lycopersici* toxins (AALs) (EFSA, 2011). *Alternaria alternata* (Fr.) Keissl. is the most important and widespread *Alternaria* species, both in terms of its wide biological activity (pathogen, saprophyte, etc.) and host distribution, and mycotoxin production and diversity (Barkai-Golan, 2008; Bottalico & Logrieco, 1998; Logrieco et al., 2009; Pinto & Patriarca, 2017; Tunalı et al., 2023). *Alternaria* spp. can produce many different secondary metabolites at different stages of pathogenicity and these are defined as host-specific toxins (HSTs) and non-HSTs (Berestetskly, 2008; Friesen et al., 2008). Both groups of toxins are considered to be a "virulence factor" of *Alternaria* species, especially in terms of plant pathogenicity (Andrew et al., 2009). *Fusarium* species produce three most important classes of mycotoxins namely: trichothecenes, zearalenone (ZEN), and fumonisins (FBs). Among the fungi isolated from wheat grain, *F. graminearum* and *F. poae* are also important pathogens. These fungi are among the leading agents causing head blight disease in small grains. Although several species have now been described within the clade, *F. graminearum* sensu stricto remains the most economically important toxigenic species in the genus, as it is the most frequent cause of Fusarium head blight of small grains and Gibberella ear rot of maize throughout most of the world. Several mycotoxins with different chemical structures have been reported to be associated with health problems in humans and animals (Munkvold et al., 2021). *Fusarium graminearum* can produce multiple mycotoxins, but production of the DON during the development of Fusarium head blight of cereals is most significant. *F. graminearum* is the species from which DON was first characterized (Vesonder et al., 1973, Yoshizawa & Morooka, 1973).

In this study, fungal flora was examined nine months after harvest. Isolates belonging to *Penicillium* and *Aspergillus* genera were also identified as a result of the examination, while in a study, the flora six months later was compared with the flora immediately after harvest. Analyses of the mycoflora revealed that in the tested varieties of grains at harvest, field fungi were overwhelmingly predominant constituting more than 90 % of the total number of species. *Alternaria alternata* was most predominant followed by other field fungi, *Curvularia pallescens, Cladosporium herbarum, B. sorokiniana* and species of *Fusarium* spp. and sterile fungi. The number of field fungi was found to decrease significantly with prolonged storage in all cases. The percentage of *Aspergillus* and *Penicillium*, on the other hand, which were present only occasionally at harvest, showed a continuous increase during the storage period (Ghosh et al., 1981).

5. Conclusion

As a result of this study, it was determined that a large number of fungal species were present in the fungal flora of wheat seeds nine months after harvest. With this study, we think that it would be useful to examine what kind of differences in the fungal flora in producer warehouses immediately after harvest and after certain periods of time after harvest. As a matter of fact, there are studies on this subject in the world. In addition, both endophytic fungi that can be used in biological control and toxigenic and saprophytic fungi obtained as a result of the research should be emphasized.

Conflict of Interest

The authors declare that they have no conflict of interest.

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