



Isolation and identification of the fungi associated with wheat, barley, and maize cereals

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Received: 29/6/2026 Revised: 20/7/2026 Accepted: 28/7/2026 Publication: 29/8/2026

ABSTRACT

The purpose of this study was to isolate the fungi correlating with some cereals of the Poaceae family. Three types of cereals (wheat, barley, and maize) were collected from the local market.. The isolation was carried out in two ways: standard blotter and agar plate method, sterilized seeds with sodium hypochlorite, and unsterilized cereals were used. The germination percentage and infection percentage vary depending on the type of cerea. When the agar plate method was used with wheat cereals, the infection percentage reached 100% for both sterilized and non-sterilized cereals; for barley, the percentage were 100% and 80% for non-sterilized and sterilized cereals, respectively. In contrast, sterilized maize cereals were completely free of fungi when the two aforementioned isolation methods were employed. The results of this study showed that when cereals were used directly without sterilization, fungi known as surface fungi were found on their outer surfaces; however, after sterilization, fungi known as endophytic fungi were detected. *Aspergillus sp.*, *Alternaria sp.*, *Fusarium oxysporum*, *Rhizopus stolonifer*; *Penicillium chrysogenum*, *Mucor piriformis* ,and *Saccharomyces cerevisiae* are the most important fungi isolated from the tested cereals. The moisture content of the tested cereals was also measured to determine the appropriate storage period for each type; the results indicated moisture levels of 11% for wheat, 11.9% for barley, and 13% for maize. These percentages indicate that the suitable storage period for these cereals ranges from 6 to 12 months.

Keywords: Isolation, Identification, Wheat, Barley, Maize.

1. Introduction

Achieving high plant productivity requires the use of high-quality cereals; however, fungi play a significant role in reducing cereal quality, leading to substantial economic losses in crop production (Youssef, *et al.*, 2008). The contamination of agricultural crops and cereal in particular is the primary cause of disease within food chains for humans and animals (Soliman, 2003). Cereal goods supply vital nutrients for human health; however, product quality and agricultural safety are crucial due to rising rates of chronic diseases linked to the consumption of contaminated materials (Al-Defiery and Merjan 2015).

Direct plating is considered one of the best methods for identifying the type of fungal colonization and is highly useful for assessing cereals quality, considering that most fungal microflora contamination in agricultural crops is caused by fungus. *Aspergillus*, *Fusarium* and *Penicillium* are the fungus genera that can poison both humans and animals (Soliman,

2003 Youssef, *et al.*, 2008). Many agricultural crops' microbial flora frequently includes these fungus (Palumbo, *et al.*, 2008).

The fungus genera *Alternaria*, *Aspergillus* and *Fusarium* have been found to contaminate wheat in varied degrees (Tournas and Katsoudas, 2008). Microorganisms are transferred to cereals in various ways, the most common being via the surfaces of plant residues during harvesting, transport, storage, and processing. (Youssef, *et al.*, 2008). Numerous studies have been conducted worldwide on cereals contamination (Almeida, *et al.*, 2000; Al-Defiery and Merjan, 2015; Marei, *et al.*, 2016 Soliman, 2003).

Fungal contamination of barley is a significant issue due to the ability of fungi to proliferate during storage; Sagir and Yildiz (2004) determined the growth rates of the fungi *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium canescens* on barley. Maize (*Zea mays* L is a major cereal crop that dominates global agriculture (Al-Defiery and Merjan, 2015). Maize cereals serve as a suitable medium for fungal growth and the production of dangerous mycotoxins that can harm human and animal health (World Health Organization, 2006). Therefore, this research aimed to isolate and identify fungi from three types of cereals (wheat, barley, and maize).

2. Materials and Methods

2.1. Collecting of Cereals Samples :

The cereals used in the study, which belong to the *Poaceae* family, are wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and maize (*Zea mays* L.). The cereals were collected from a local market in the study area.

2.2. Determination of Cereals' Moisture Content:

The moisture content of cereals can be determined using the oven-drying method described by the International Seed Testing Association (ISTA 2005).

The moisture content of the cereals is determined as follows: the container and its lid are weighed, and the weight is recorded (W1). Twenty grams of the cereal sample are ground and placed into the containers with their lids; the assembly is then weighed, and the weight is recorded (W2). With the lids removed, the containers are placed in an oven set at 105°C for 24 hours. At the end of the drying period, the lid is placed back on the container. After being moved to a desiccator, the containers are left to cool for forty-five minutes. The containers' dry weight, which includes the samples, is noted (W3). The following formula is used to determine the cereals' moisture content:

$$\text{SMC (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

SMC = Seed moisture content; **W1** = weight of a container with lid; **W2** = weight of container with lid and sample before drying; **W3** = weight of container with lid and sample after drying.

2.3. Assay of the mycota associated with cereals :

Microbiological examination of cereal-borne fungi was conducted using the standard blotter method (ISTA, 1976) and the agar plate method (Muskett, 1948).

2.3.1. Standard Blotter method

The cereal samples are immersed in a 6% sodium hypochlorite solution for one to two minutes, then washed three times (for two minutes each time) with distilled water.

Subsequently, the cereal samples, whether sterilized or non-sterilized, are put in filter paper-lined Petri dishes at a rate of ten cereals per dish. Both sterilized and non-sterilized cereals were used in three replicates of the experiment, and the dishes were stored at room temperature for seven days.

2.3.2. Agar plate method

Twenty milliliters of Potato Dextrose Agar (PDA) medium were added to petri dishes containing ten cereals, including both surface-sterilized and non-sterilized samples. For seven days, the dishes were incubated at 25°C. The samples were inspected under a microscope to look for related fungi after incubation. The following references were used to classify and identify the fungi (based solely on morphological characteristics, both macroscopic and microscopic): (Raper and Fennel, 1977; Domsch *et al.*, 1981; Nelson *et al.*, 1983 and Pitt, 1985). The germination percentage and the percentage incidence were recorded by the following formulas:

cereal germination percentage (%) = Number of cereal germinated/ Total number of cereal used × 100

Percentage incidence (%) = Number of cereal bearing fungal colonies/ Total number of cereal examined × 100

3. Results and Discussion

Based on oven-dry weight basis, the moisture content of the cereal samples (wheat, barley, and maize) varied from 11% to 11.9% and 13.6%, respectively. These variations in cereal water content are considered normal and are attributed to differences in storage conditions and the maturity of the crops at the time of harvest. Because moisture level is the most important factor influencing the rate of cereal deterioration and has a significant impact on storage longevity, it is crucial to ascertain the moisture content before storage in order to precisely estimate each sample's possible shelf life. The lower the moisture content of the cereals, the lower the rate of deterioration; moisture levels should range between 5% and 14%. cereals are suitable for short-term storage (6 to 12 months) if their moisture content is between 11% and 13%, whereas they are suitable for long-term storage (1 to 3 years) if the moisture content is between 8% and 10%. Also, microorganisms that infect stored cereals thrive when seed moisture content ranges between 10% and 30%; exposing the cereal to air and sunlight helps reduce moisture levels, thereby lowering the incidence of fungal diseases. (Agrios, 1997).

Table 1 summarizes the results of fungal isolation from the tested cereals. Overall, a total of 11 fungal species belonging to 7 genera were isolated and identified from three types of cereals: wheat, barley, and maize. Two *Alternaria* species were among the fungus that were isolated from the wheat (*A. alternata* and *A. tenuissima*); four *Aspergillus* species (*A. flavus*, *A. niger* and *A. clavatus* and *A. terreus*); *Fusarium oxysporum*; *Rhizopus stolonifer*; *Penicillium chrysogenum*; *Mucor piriformis* *Saccharomyces cerevisiae*. The results showed that the isolation of fungi from cereals varies according to the plant type.

Aspergillus was the most prevalent fungus in wheat cereallss, followed by *Rhizopus stolonifer* and *Alternaria alternata*, while *Fusarium* was less common. These results align with the findings of Kacaniova and Tancinova (2001), who identified that the 24 genera most commonly isolated from wheat were *Aspergillus*, *Alternaria*, *Penicillium*, and *Rhizopus*.

Species of *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Mucor piriformis*, and *Rhizopus* were found to be predominant among the mycoflora of barley cereals collected from various locations worldwide. (Soliman, 2003 and Abramson, 1999). *Saccharomyces cerevisiae* and *Aspergillus flavus* and *Alternaria tenuissima* and *Fusarium* were detected in maize cereals. Results of fungal isolation from surface-sterilized maize cereals demonstrated that no fungi were detected on the filter paper or on the potato dextrose agar medium. Fungal populations were observed in freshly harvested maize cereals collected from regions in Brazil in a study conducted by Almeida (2000). Furthermore, It was shown that the fungus *A. flavus* and *F. moniliforme* are responsible for the most severe issues with maize contamination (Soliman, 2003 and Marin, *et al.*, 1998).

Cereal germination percentage used in the study are presented in Table 2. The observed decline in cereal germination and viability may be attributed to storage conditions, the impact of fungi isolated from the cereals, or toxins produced by *Aspergillus* fungi; indeed, most fungi responsible for grain deterioration during storage belong to the genera *Aspergillus* and *Penicillium*, and other species also compromise cereal viability. (Agarwal and Sinclair, 1987)

In this study, using agar plates instead of the blotter method resulted in a higher percentage of cereal-borne fungus being found. This could be explained by the capacity of agar plates to sustain the development of a variety of fungal species or by the effectiveness of the nutritional medium in increasing the colonization rate of fungi found both inside and on the surface of the cereal. The findings show that there is a difference between the two isolation techniques used in terms of the prevalence of the fungal community-that is, the percentage of occurrence.

The results of this study showed that certain species or genera were more prevalent, while others were observed sporadically or were entirely absent. It has been noted that treating cereals with sodium hypochlorite reduces or eliminates externally borne fungi (Jaffe, 1968). These fungi associated with the cereal surface are known to cause cereal deterioration by reducing germination capacity; however, such deterioration may also be attributed to cereal-borne fungi that are not limited to the surface but are also present within the tissues of the cereal coat, embryo, and cotyledons (Jayaweera *et al.*, 1988).

Table 1: Shows the species of fungi isolated from the tested cereals

Cereals tested Method of isolation		Wheat	Barley	Maize
Standard blotter method	UC	<i>Aspergillus niger</i> <i>Aspergillus clavatus</i>	<i>Aspergillus niger</i> <i>Fusarium oxysporum</i> <i>Penicillium chrysogenum</i>	<i>Fusarium oxysporum</i>
	SC	<i>Aspergillus clavatus</i>	<i>Aspergillus niger</i> <i>Rhizopus stolonifer</i>	
Agar plate method	UC	<i>Rhizopus stolonifer</i>	<i>Aspergillus niger</i> <i>Aspergillus terreus</i> <i>Penicillium chrysogenum</i> <i>Alternaria alternata</i> <i>Mucor piriformis</i>	<i>Saccharomyces cerevisiae</i> <i>Aspergillus flavus</i> <i>Alternaria tenuissima</i>
	SC	<i>Aspergillus flavus</i> <i>Fusarium oxysporum</i> <i>Alternaria alternata</i>	<i>Alternaria tenuissima</i> <i>Fusarium oxysporum</i>	

UC- Unsterilized cereal, SC- Sterilized cereal

Table 2: Shows the Cereal germination percentage and the percentage of incidence.

Cereals tested		Wheat		Barley		Maize	
Method of isolation		GP (%)	Incidence (%)	GP (%)	Incidence (%)	GP (%)	Incidence (%)
Standard blotter method	UC	70	60	70	40	30	10
	SC	60	10	90	20	10	0
Agar plate method	UC	60	100	80	100	10	60
	SC	50	100	80	80	60	0

GP- germination percentage, UC- Unsterilized cereal, SC- Sterilized cereal



Unsterilized wheat cereals



Sterilized wheat cereals

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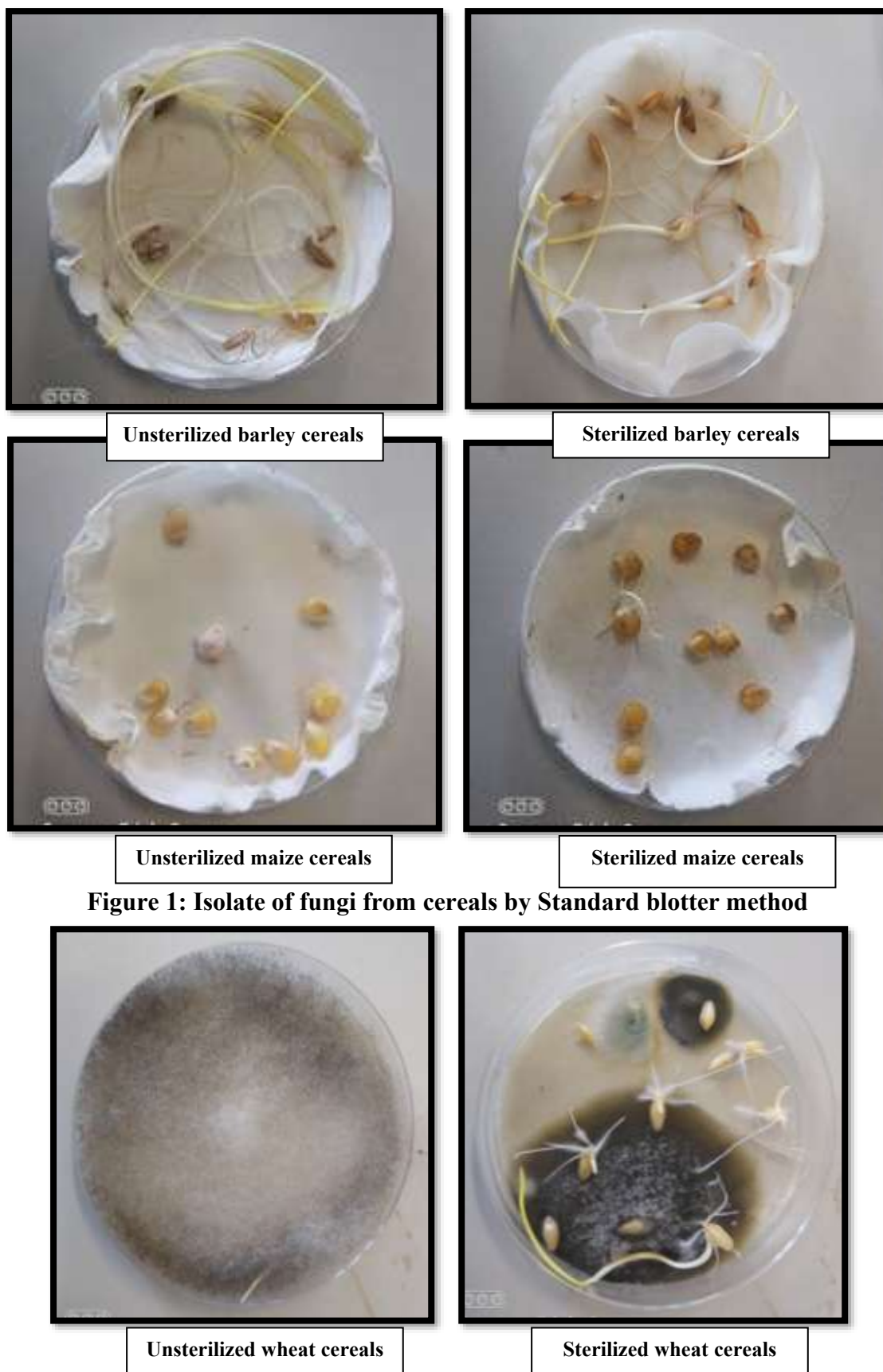


Figure 1: Isolate of fungi from cereals by Standard blotter method

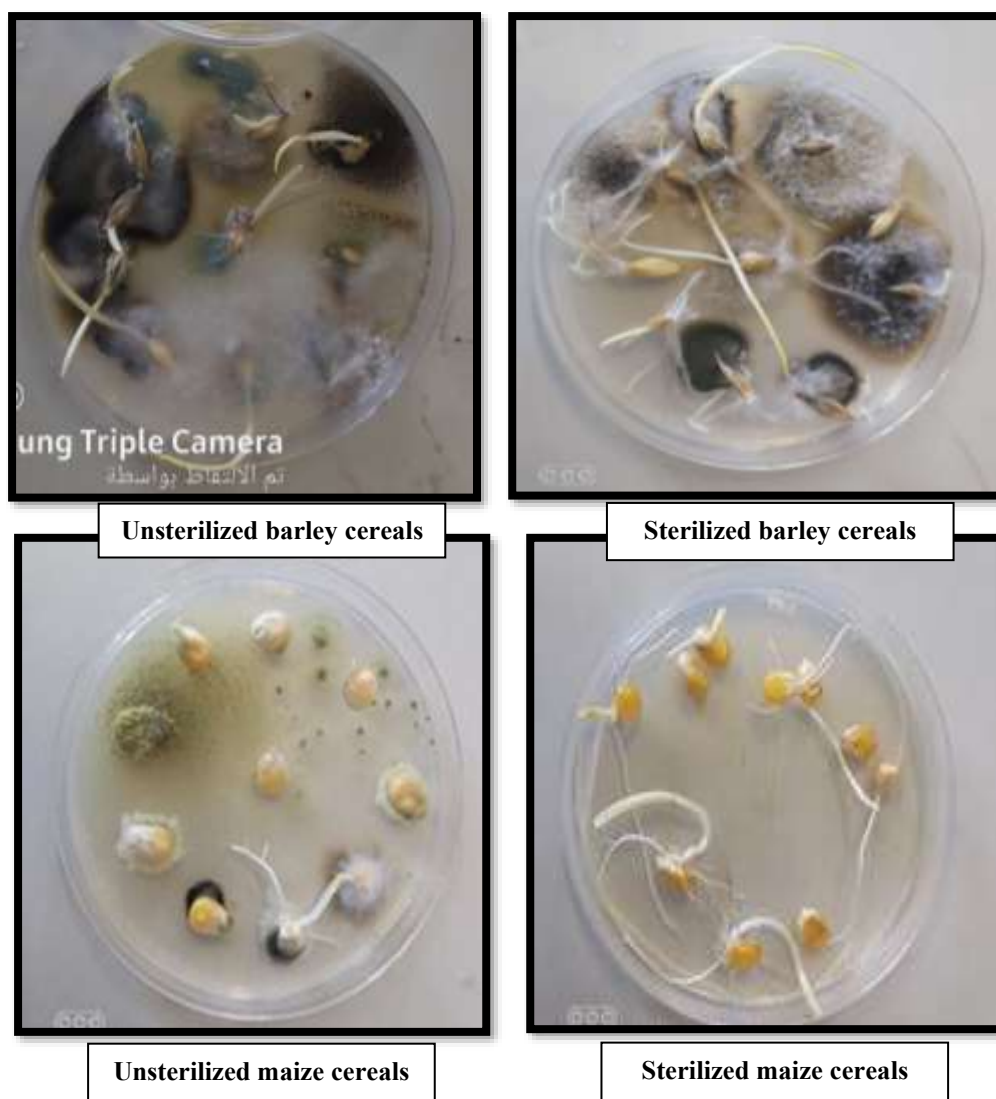


Figure 2: Isolate of fungi from seeds by Agar plate method

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