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Mycoflora of Wheat Grains in the Main Production Area in Kerman Province, Iran

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ABSTRACT

Kerman province is an important wheat growing area where harvested wheat is stored in silos every year. Wheat grains are often contaminated with many kinds of fungi before or after harvesting, which are transferred to silos. Some contaminations may develop in silos, and decrease food quality, packing and marketing of wheat. In this study, mycoflora of stored grains were investigated. Several samples were randomly collected from five silos at different locations in Kerman province. For mycological analysis, the moist chamber method and agar test were used. After mycelial growth or sporulation, the fungi were transferred to potato dextrose agar media. Subsequently, single-spore culture was established on 2% distilled water agar. As result, the fungi were isolated and identified as following: *Alternaria alternata, Ulocladium alternariae, Aspergillus flavus, A. niger, Chaetomium globosum, Fusarium proliferatum, Cladosporium cladosporioides, Rhizopus* spp. *Penicillium* spp

Key Words: Kerman; Wheat; Grain; Mycoflora; Contamination

INTRODUCTION

Wheat is one of the most important cereals and main source of food in Iran and many different countries. Kerman province is an important wheat growing area where most harvested wheat is stored in silos every year. Wheat grains are often contaminated with many kinds of fungal agents before or after harvesting and these grains are transferred to silos. Some contamination may develop in silo condition and decrease food quality, packing and marketing of wheat. A lot of information is available regarding the mycoflora of harvested wheat grain, including German (Weidenboner *et al.*, 1996); Nigeria (Adisa, 1994); Poland (Klyszejko *et al.*, 2005) and Russia (Kroiakova *et al.*, 1989).

In Iran little information is available on the fungal species associated with wheat grain and their distribution in the main production area. Saberi *et al.* (2004) showed for the first time that wheat grain of Markazi province, Iran contaminated with *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Penecillium* spp. and *Ulocladium* spp. The objective of this study was to identify the fungi associated with wheat grain harvested in an extensive region of the main production area in Kerman province, Iran. Moreover, study of presence or absence of toxigenic species such as *Aspergillus flavus, A. niger, Alternaria alternata* and allergic species such as *Ulocladium consortiale, Cladosporium herbarum* which can be dangerous for people and farm livestock was felt to be necessary.

MATERIALS AND METHODS

Wheat grain sampling. A total of 30 samples (1.5-3 Kg) of wheat grains harvested in 2002-2003 were collected from five silos containing 20000-40000 tons of wheat located in different areas of Kerman province including: Jiroft, Kahnoj, Zarand, Mahan and Ravar.

Mycological analysis. The preparation of the samples and analysis were carried out according to the methods described by Harrigan and McCance (1976). At first, sub-samples of grain (each ca. 10 g) from each sample were surface sterilized for 1min with 1% sodium hypochlorite solution. For mycological survey of grain sample, the moist chamber method was used: 50 seeds were placed in petridishes (diameter 10 cm) on a moist filter paper replicated thrice at 20°C for 7 days. In addition, 50 seeds per sub sample were plated onto potato dextrose agar medium. The plates were incubated at 25°C for 5-7 days. The developing fungal colonies were counted directly and where several fungi were isolated from a single grain, all the colonies were recorded, and different species sub-cultured onto PDA medium. Agar test was also used in order to investigate the growth rate of fungi on the relatively poor medium WA.

Identification of fungal species. Isolates were sub-cultured as single spore by dilution plating and then identified by using the morphological criteria of Ellis (1976), Hanlin (1990) and Raper and Fennell (1965). For, identification of *Fusarium* spp. subcultures were made on SNA (Spezieller Nahrstoffarmer agar), CLA (carnation leaf agar) and PDA (potato dextrose agar) incubated at 25°C for 5-7 days. Final identification was made following Leslie and Summerell (2006) as well as Nelson *et al.* (1983) for *Fusarium* spp.

RESULTS AND DISCUSSION

The results of mycological tests indicate that the grains stored in the silos of Kerman province are strongly contaminated with micromycetes, consisting of 7 fungal species from 8 genera. The typical epiphytic fungi (*Penicillium, Aspergillus, Cladosporium),* the so-called storage fungi- xerophytes (*Penicillium, Aspergillus,*

Rhizopus) and the pathogen (*Fusarium*) were represented. Some of them like, *A. flavus* and *Alternaria alternata* normally produces aflatoxin and alternariols, a very toxic and carcinogenic toxin that is harmfull for people and farm livestock As result, the fungi, which were isolated and identified as: *Alternaria alternata*, *Ulocladium alternariae*, *Aspergillus flavus*, *A. niger*, *Chaetomium globosum*, *Fusarium proliferatum*, *Cladosporium cladosporioides*, *Rhizopus* spp. , *Penicillium* spp. Descriptions of many fungi are as follows:

Ulocladium alternariae. Conidiophores up to 100 x 4 - 7 µm, pale golden brown, conidia mostly broadly ellipsoidal, golden brown, with1-5 transverse and 1or more longitudinal or oblique septa, 20-33 x 15 µm (Fig. 1). This result confirms the study conducted by Ellis (1976).

Alternaria altrnata. Conidiophores simple or branched, smooth, up to 50 long, 3 - 6 µm thick with 1 or several conidial scars. Conidia formed in long, often branched chains, obclavate, ovoid or ellipsoidal, pale to mid golden brown, with up to 8 transverse and usually several longitudinal septa, overall length 22 – 60 x 9 – 3 µm thick in the broadest part (Fig. 2). This result is similar to the study conducted by Ellis (1976).

Fig. 1. ***Ulocladium alternariae***

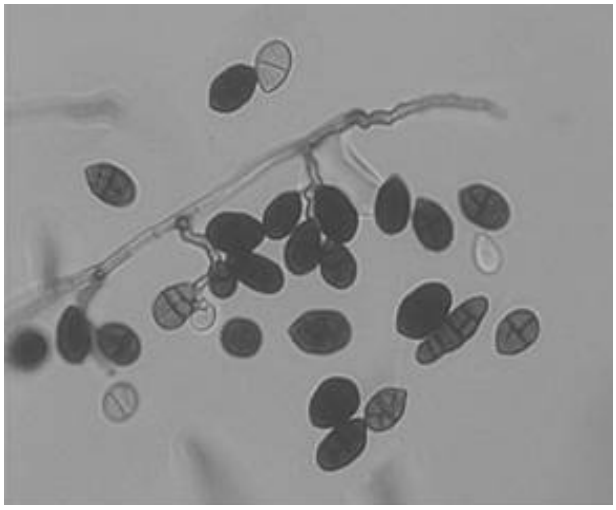

Fig. 2. ***Alternaria alternata***

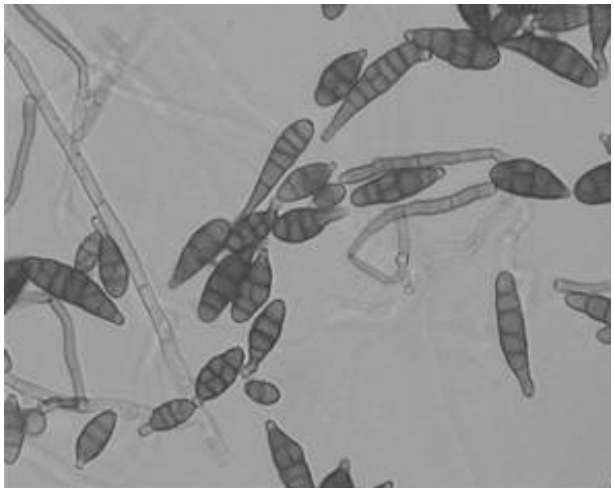

Fig. 3. ***Chaetomium globosum***

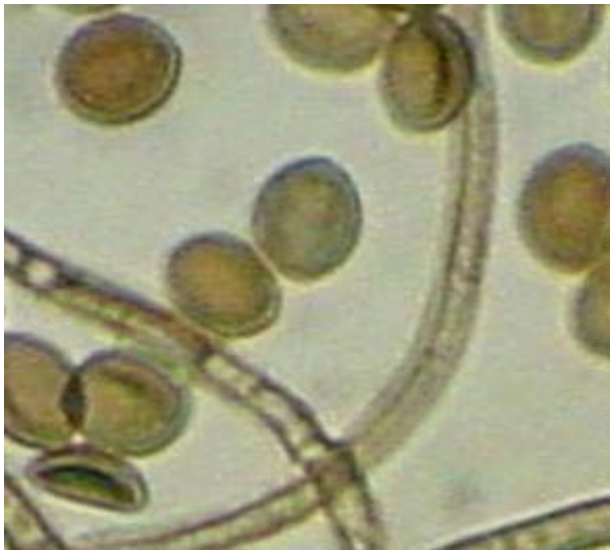

Fig. 4. ***Fusarium proliferatum***

Chaetomium globosum. Ascoma an ostiolate perithecium, superficial, single to gregarious, globose, clothed with hairs of various shapes. Asci clavate, 4-8 spores with evanescent walls. Ascospore 1-celled, light olive to dark brown (Fig. 3). Hanlin (1990) reported similar results.

Fusarium proliferatum. Colonies fast growing, reaching 7.5-8 cm in 8 days at 25°C on PDA. Primary conidiophores initially unbranched, later more or less loosely ramose arising laterally on hyphae in aerial mycelium. The isolate of *F. proliferatum* on ClA produced chain of single-celled microconidia but sometimes with a septum mostly on

polyphialides and orange spordochium (Fig. 4).

Microconidia measured 4.8 - 12.4 × 2.8 - 3.3 µm. Dimension of macroconidia with 3 septa were 25-41×1.9-2.8µm. These results confirm the study carried out by Leslie and Summerell (2006).

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