# A comparative study of mycoflora of Iranian and imported soybeans

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***Abstract:*** **The natural occurrence of fungal contamination was evaluated in stored soybeans in different agro-ecological zones. Of 30 samples examined, fungal contaminations were positive in 25.9 percent and 74.1 percent of Iranian and imported soybeans ($p<0.05$). The total fungal CFU/g counts were calculated $6.3 * 10^2$ in Iranian and $18 * 10^2$ in imported samples. The most frequent isolated fungi from soybeans originated from Iran and imported were *Aspergillus spp*. (59.7, 58.6 percent), *Penicillium spp*. (26.8, 27.3 percent), and *Fusarium spp*. (13.5, 14 percent), respectively. Soybeans with a high incidence of diverse species of fungi to need for proper surveillance and monitoring for the prevention of fungal and mycotoxin contaminations.**

**Key words:** soybean, mycoflora, Iran.

## Introduction

During the last 25 years, the frequency of life-Fungal growth on foods and feedstuffs is one of the major threats to human and animal health (Benkerroum and Tantaoui-Elaraki, 2001). In general, foods and feedstuffs are excellent substrates enhancing fungal growth, so fungi permanently contaminate them. Up to now, more than 100000 fungal species are considered as natural contaminants of agricultural and food products (Kacaniova, 2003). A majority of the toxic species belongs to the genera Aspergillus, *Penicillium*, and *Fusarium* (Kaushal and Sinha, 1993). Besides their negative impacts on nutritional and organoleptic properties, fungi can also synthesize different mycotoxins. The effects of mycotoxins on animals include hepatotoxicity, nephrotoxicity, immunotoxicity, oncogenesis and genotoxicity (Dierheimer, 1998; Oswald, 1998). According to Leibetseder (1989), 30 to 40 percent of existing fungi can produce toxic substances under favorable conditions. The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage (Huis in´t Veld, 1996; Pitt and Hocking, 1997; Kubátová, 2000). Soybeans are recognized by nutritionists as high-quality, very digestible feed ingredients and excellent sources of protein, lipids (oils), minerals and vitamins for addition to the diet of most farm animals (Kacaniova, 2003). Regarding investigator reports, Aspergillus, *Fusarium*, and *Penicillium* have been detected in all agricultural seeds (Moharram *et al*., 1989; El-Kady and Youssef, 1993; Abarca *et al*., 1994). Despite great attention that has been paid to the study of toxigenic fungi and their mycotoxins in various foods and feedstuffs, also, it is well established that fungal and mycotoxin contaminations of animal feedstuffs, especially poultry, can develop sanitary disturbances and mortality among the birds and secondary contamination of the human consumer via both eggs and poultry meat as well as the consumption of soymilk

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**Table 1: Data analysis of fungal colony count in Iranian and imported soybeans in 2006.**

| Data / Origin | Petri dish (No.) | Minimum | Maximum | Mean | Standard deviation | P value |
|---|---|---|---|---|---|---|
| Iranian soybean | 75 | 31 | 99 | 63 | 17.51 | < 0.05 |
| Imported soybean | 75 | 141 | 251 | 180 | 25.46 | |

(Pennington, 1986). Since soybeans have been broadly used in poultry diets in Iran, therefore it is imported from other countries. The naturally fungal contamination of soybean based-feedstuffs from Iran had not been studied to date. The goal of this study was to identify and count of moulds, especially toxigenic fungi, in the samples of imported and native soybean samples.

## Materials and Methods

The samples of soybean were taken from agricultural companies in the year 2006. The total amount of the tested samples was 30; 15 from Iran and 15 from imported soybeans. One hundred grams of each sample were ground with a mortar and pestle in a solution containing glycerol, sucrose, KCI and tris buffer, pH 7.0. Then, 1 gram of each ground sample was transferred into tube, added 9 ml of 0.1 percent peptone (Merck, Darmstadt, Germany), and shook vigorously for 15 seconds, and incubated at room temperature for 30 minutes. For the determination of fungal colony-forming units per gram (CFU/g), 1 ml of supernatant (dilution 1:10) was transferred into petri dish, added 10 ml of dichloran rose-bengal chloramphenicol (DRBC, Sigma, St. Louis, USA) agar, and the mixture was shaken slowly for 10 seconds. Five replicate plates were used and incubated for 5 days at 25°C in a dark chamber. Total fungal CFU/g counts in each sample were determined after 5 days of incubation. Subsequently, the colonies were exactly isolated and sub-cultured on slant potato dextrose agar (PDA, Merck, Darmstadt, Germany) and sabouraud glucose agar (SGA, Merck, Darmstadt, Germany) media. *Fusarium* species were isolated, transferred onto spezieller nahrstoffarmer agar (SNA, Difco, Darmstadt, Germany) and incubated at 25°C for 7 days. Final identification of *Fusarium* species was conducted according to Nelson *et al* (1983) method. Also, Aspergillus species were identified using PDA and czapek-dox agar (CZA, Merck, Darmstadt, Germany) media and according to Raper and Fennell (1965) method. The other genera were identified using PDA and SGA media.

Unpaired Student's t test was performed using SPSS software (Version 13.0) and differences were considered significant at $p< 0.05$.

## Results

Of 30 samples examined, 15 samples were from Iran and the rest belong to imported soybeans. Each sample was cultured on 5 petri dishes. In this study, 4725 (25.9 percent), and 13500 (74.1 percent) fungal colonies were isolated from Iranian and imported samples, respectively. Significant difference was observed between the frequency of fungal isolates of Iranian and imported soybeans ($p<0.05$) (Table 1). The amount of CFU /g in Iranian and imported samples were calculated approximately $6.3 \times 10^2$ and $18 * 10^2$, respectively. The identification of fungal isolates obtained revealed that they belonged to 9 different genera. As shown in Table 2, *Aspergillus spp*. and *Penicillium spp*. were the most predominant fungal genera in all soybean samples. More than 86 percent of the samples were found to be infected with species of these two genera. The isolated *Aspergillus* species were as follows: *Aspergillus flavus* (49.2 percent, 38.6 percent), *A. niger* (35.5 percent, 62.3 percent), *A. ochraceous* (21.3 percent, 38.9 percent), *A. oryzae* (18.7 percent, 19 percent), *A. parasiticus* (12.1 percent, 22.3 percent), *Aspergillus spp*. (16.5 percent, 17.2 percent) and *A. fumigatus* (4.1 percent, 13.9 percent) in Iranian and imported soybeans, respectively. The genus *Penicillium* was also detected in many samples, but with lower incidence about 21.1 percent. Mycological analysis also

**Table 2: The relative frequency of toxigenic fungal isolates from Iranian and imported soybeans in 2006.**

| Isolate | Origin | |
|---|---|---|
| | Imported soybean (percent) | Iranian soybean (percent) |
| *Aspergillus flavus* | 38.6 | 49.2 |
| *Aspergillus niger* | 62.3 | 35.5 |
| *Aspergillus ochraceous* | 38.9 | 21.3 |
| *Aspergillus parasiticus* | 22.3 | 12.1 |
| *Aspergillus oryzae* | 19 | 18.7 |
| *Aspergillus spp.* | 17.2 | 16.5 |
| *Penicillium* | 92.4 | 68.7 |
| *Fusarium proliferatum* | 31.8 | 17.5 |
| *Fusarium solani* | 4.3 | 11.7 |
| *Fusarium oxysporum* | 8.7 | 5.5 |
| *Fusarium spp.* | 2.7 | 0 |

revealed the presence of *Alternaria*, *Rhizopus*, *Mucor*, *Fusarium*, *Cladosporium*, *Scopolariopsis* and *Curvularia* in Iranian and imported soybeans. A difference was observed between the frequency of storage mycoflora including *Aspergillus* and *Penicillium* species in Iranian and imported samples. The incidence of these 2 genera in Iranian samples was higher than imported ones.

## Discussion

Soybean has been broadly used in human alimentation and feedstuff preparation for livestock. This study deals with investigations on fungal flora of Iranian and imported soybeans under natural condition. The results revealed 25.9 percent ($6.3 * 10^2$ CFU/g) and 74.1 percent ($18 * 10^2$ CFU/g) fungal colonies isolated from Iranian and imported samples, respectively ($p<0.05$). The identification of fungal isolates obtained showed that they belonged to 9 different genera. As shown, *Aspergillus spp.* and *Penicillium spp.* were the most predominant fungal genera (more than 86 percent) in all soybean samples. The isolated *Aspergillus* species were as follows: *Aspergillus flavus* (49.2 percent, 38.6 percent), *A. niger* (35.5 percent, 62.3 percent), *A. ochraceous* (21.3 percent, 38.9 percent), *A. oryzae* (18.7 percent, 19 percent), *A. parasiticus* (12.1 percent, 22.3 percent), *Aspergillus spp.* (16.5 percent, 17.2 percent), and *A. fumigatus* (4.1 percent, 13.9 percent) in Iranian and imported soybeans, respectively. The genus *Penicillium* was also detected in many samples, but with lower incidence about 21.1 percent. Mycological analysis also revealed the presence of *Alternaria*, *Rhizopus*, *Mucor*, *Fusarium*, *Cladosporium*, *Scopolariopsis* and *Curvularia* in Iranian and imported soybeans. The occurrence of above reported fungi is limited, because *Aspergillus* and *Penicillium* species predominate in all kinds of cereal meals under any storage conditions. They actively grow on stored seeds and have antagonistic effect on other fungal growth, thus, progressively eliminate intermediate and field mycoflora such as *Fusarium*, *Alternaria*, *Cladosporium* and Trichoderma (Kohler, 1981; Lee *et al*., 1986). In a study conducted on soybean mycoflora by Moharram *et al* (1989), among the 73 fungal species, *A. flavus*, *A. niger*, *A. fumigatus*, A. terreus, A. flavipes, *Mucor* circinelloides, Scopulariopsis brevicalis, *Penicillium* chrysogenum, *Fusarium* moniliforme and *Rhizopus* stolonifer were found to be common. In general, all genera identified in this study have been reported to occur naturally on food products (Kurata and Ueno, 1984; Marsilio and Spotti, 1987). It is mentioning that the frequency of *Aspergillus* and *Penicillium* species in Iranian samples was higher than that imported ones. Considering a high incidence of fungal contamination of imported soybeans, it seems that the difference in climate conditions of two regions, and also, the traditional methods of handling grains

during harvesting in the field, drying process in relevant country, and transferring it to other countries lead to mechanical damages of grains. In this condition, broken, and ground grains are more vulnerable to fungal attack than whole grains. On the other hand, this contamination could be due to long-term storage of imported soybeans in the poor environmental conditions including high moisture and temperature in borderlines and barns in Iran. Soybeans stored for long-time periods are more vulnerable than freshly harvested soybeans. Insects may also contribute to deteriorating the grains rapidly and increasing soybean mycoflora during long-term storage (Bilgrami and Choudhary, 1990). In general, the lack of proper storage facilities induces fungal contamination and accumulation of mycotoxins during the post-harvest period. Therefore, the proper handling, transferring, and storing of soybeans during the post-harvest phase is crucial to preserve grains for longer periods. We suggest that monitoring fungal contaminations and mycotoxins in imported soybeans can be simplified using predetermined profiles of soybean mycoflora for each exporting country.

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# یک مطالعه مقایسه‌ای از فلور قارچی سویاهای ایرانی و وارداتی

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## چکیده

**میزان آلودگی قارچی به صورت طبیعی در سویاهای انبار شده در مناطق مختلف آب‌وهوایی در ایران مورد ارزیابی قرار گرفت. از ۳۰ نمونه آزمایش شده، آلودگی‌های قارچی در ۲۵/۹ درصد از سویاهای ایرانی و ۷۴/۱ درصد از سویاهای وارداتی مثبت بودند ($p<0/05$). تعداد کلنی‌های قارچی در هر گرم از نمونه‌های ایرانی در حدود $۶/۳\times۱۰^{۲}$ و در نمونه‌های خارجی $۱۸\times۱۰^{۲}$ محاسبه شدند. فراوان‌ترین قارچ‌های جداشده از سویاهای با منشاء ایرانی و وارداتی به ترتیب شامل گونه‌های آسپرژیلوس (۵۹/۷ و ۵۸/۶ درصد)، گونه‌های پنی‌سیلیوم (۲۶/۸ و ۲۷/۳ درصد) و گونه‌های فوزاریوم (۱۳/۵ و ۱۴ درصد) بودند. آلودگی بالای سویاها به گونه‌های مختلف قارچی نیازمند مراقبت صحیح و نظارت بهداشتی جهت جلوگیری از آلودگی‌های قارچی و توکسین‌های قارچی دارد.**

**واژه‌های کلیدی:** سویا، فلورقارچی، ایران.

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