http://www.trakya.edu.tr/Enstituler/FenBilimleri/Dergi/net/index.htm
Trakya Univ J Sci, 4(2): 165-168, 2003
ISSN 1302 647X
DIC: 86MKNS12030104

Original Article/Orijinal Makale

FEEDING SOYBEAN COLONIZATION BY MICROSCOPIC FUNGI

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Received : 03.04.2003
Accepted : 15.05.2003

Abstract: During the experimental period of months March, August, November and January of the years 2002 and 2003, the amount and generic composition of microscopic fungi in soybean heve been monitored. Sampling was done in five takings (25 samples). The aim was to monitor changes in microflora of feedstuff soybean. The highest contamination was detected in the second taking represented by 10^2 to 10^3 CFU (colony forming units) of microscopic fungi per 1 gram, followed by first taking in March ($2.67.10^3$ CFU.g^{-1}). From the analyzed samples, 26 species belonging to 13 genera of microscopic fungi were isolated and identified. Among the isolated genera, *Aspergillus* and *Penicillium*, belonged to the most dangerous fungi, occuring in 67 - 100 % and 50 - 88 % of the samples tested, respectively. Stored cereals can be seriously damaged by these genera owing to grain moulding as well as by production of mycotoxins and allergenes. Species determined as *Aspergillus fumigatus, Aspergillus flavus* etc. can contaminate and impair the feedstuff soybean.

Key words: feedstuff soybean, microscopic fungi, genera identification.

Yem Olarak Kullanılan Soya Fasulyesinin Mikroskobik Funguslarla Kolonizasyonu

Özet: 2002 ve 2003 yılları Mart, Ağustos, Kasım ve Ocak aylarındaki deneysel periyotlar süresince, soya fasulyesindeki mikroskobik fungusların miktarı ve genel kompozisyonunu çalıştık. Örnekleme 5 defa yapılmıştır. Deney, yem olarak kullanılan soya fasulyesinin mikroflorasındaki değişmelerin çalışılmasını amaçlamıştır. En fazla kontaminasyon, ikinci örneklemede saptanmıştır, burada 1 gram için 10^2 - 10^3 CFU mikroskobik fungus saptanmış; bunu birinci örnekleme takip etmiştir; buradaki sayı ise $2.67.10^3$ CFU'dir. Örneklerin analiz edilmesiyle, mikroskobik fungusların 13 genusuna ait 26 tür izole ettik ve teşhisini yaptık. İzole edilen genuslar arasında, *Aspergillus* ve *Penicillium*'a ait olan oldukça tehlikeli funguslar vardır; bu funguslar sırasıyla % 67 - 100 ve % 50 - 88 oranında saptanmışlardır. Depodaki tahıl ürünlerine çok zarar veren sözkonusu genuslar, ciddi zararlara yol açabilir; çünkü daneler üzerindeki küfler, mikotoksin ürettikleri gibi, allerjen de olabilirler. *Aspergillus fumigatus*, *Aspergillus flavus* v.d. gibi çalışmamızda saptanan türler, yem amacıyla kullanılan soyayı kontamine edebilirler.

Anahtar kelimeler : Fungus genusları, Fungus izolasyonu ve teşhisi, Mikroskobik funguslar, Soya fasulyesi yemi.

Introduction

Soybean has been broadly used in human alimentation and feedstuff preparation for livestock. Because feedstuff in general are excellent substrates enhancing mould growing, fungi permanently contaminate them. However, the fungi occurrence is as a sulh not visible, as feedstuff appearance often remains unchanged.

The colonisation of aerial plant parts by microorganisms starts almost as soon as leaves of inflorescences are exposed to the air. Bacteria are usually the first contaminating microorganisms but they are soon joined by yeast, followed by pathogenic and saprophytic filamentous fungi. Filamentous fungi continue to develop throughout the plant's growth but especially as the plant senesces and seed ripens. Harvest profoundly disturbs the ecosystem and marks the transition from the extremes of the field environment to the relatively stable conditions of storage (Lacey, 1989).

The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage. Physiological stage of grains or sensitivity different hybrids to fungi growth are important as well (Diekman & Green, 1992; Huis in´t Veld, 1996; Pitt & Hocking, 1997; Kubátová, 2000).

The aim of this study was to monitor quantity and quality of microscopic fungi presence in the analyzed

samples of soybeans, with focus on generic identification of contaminating microscopic fungi.

Material and methods

The analyzed samples of feeding soybean were taken from agricultural companies in the years 2002 and 2003. The total amount of the tested samples of feeding soybean was 25 and the tests were carried out in March, August (two times), November and January in both years.

For determinations of fungal colony-forming units (CFU) 20 g samples of ground soybean were soaked in 180 ml sterile tap-water containing 0.02 % Tween 80 and then 30 min shaked. Dilutions (from 10^{-1} to 10^{-5}) in sterile tap water with 0.02 % Tween 80 were prepared and 1ml aliquots were inoculated on each of three plates of Czapek-Dox agar (IMUNA, Šarišské Michalany) with streptomycin (1 %, to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25°C. Total fungal CFU.g^{-1} counts in samples were determined after 10 days of incubation.

Malt agar and Czapek-Dox agar were used to isolate and identify individual genera and species. Incubation was carried out at 25°C for 5-10 days. Moulds were determined according to Pitt & Hocking (1997) and Samson et al. (1995). Dry weight was determined after drying the samples 4 hours at 105 °C.

Results

Table 1. Average numbers of the microscopic fungi (CFU.10^3.g^{-1}) of the soybeen on the Czapek-Dox agar

Sampling time	Samplings	Numbers	Statistical dates	
March	1	1.33	Average	1.634
	2	1.67	St. deviation	0.730534
	3	2.67		
	4	1.83		
	5	0.67		
August	6	0.83	Average	1.368
	7	0.17	St. deviation	1.210215
	8	2.00		
	9	3.17		
	10	0.67		
August	11	0.50	Average	0.334
	12	0.50	St. deviation	0.165015
	13	0.33		
	14	0.17		
	15	0.17		
November	16	0.17	Average	0.3
	17	0.50	St. deviation	0.137477
	18	0.33		
	19	0.17		
	20	0.33		
January	21	0.33	Average	0.466
	22	0.50	St. deviation	0.142232
	23	0.67		
	24	0.50		
	25	0.33		

Table 2. Frequency of genera incidence (%) of microscopic fungi in soybeen

Isolated genera of microscopic fungi	Number of positive samples	Frequency of incidence (%)
Absidia	6	24
Acremonium	1	4
Alternaria	5	20
Aspergillus	16	64
Cladosporium	11	44
Eurotium	8	28
Fusarium	7	32
Mucor	12	48
Mycelia	12	48
Penicillium	23	92
Rhizopus	12	48
Scopulariopsis	13	52
Trichoderma	3	12

Table 3. Incidience of species of microscopic fungi isolated from samples of soybeen

Isolated species of microscopic fungi	Number of positive samples	Frequency of incidence (%)
Absidia corymbifera	3	12
Absidia sp.	3	12
Acremonium sp.	1	4
Alternaria sp.	5	20
Aspergillus candidus	4	16
Aspergillus flavus	4	16
Aspergillus fumigatus	5	20
Aspergillus niger	4	16
Aspergillus ochraceus	2	8
Aspergillus sp.	8	32
Aspergillus sydowii	2	8
Aspergillus versicolor	2	8
Cladosporium sp.	11	44
Eurotium amstelodami	2	8
Eurotium chevalieri	2	8
Eurotium sp.	7	28
Fusarium sp.	7	28
Mucor circinelloides	3	12
Mucor hiemalis	4	16
Mucor racemosus	10	40
Mucor sp.	1	4
Mycelia sterilia	12	48
Penicillium sp.	23	92
Rhizopus stolonifer	12	48
Scopulariopsis brevicaulis	13	52
Trichoderma sp.	3	12

Discussion

Soybean extrusions and extracted grits rank among the most valuable protein feedstuff of vegetal origin. They have highly valued dietetic characteristics and are suitable for all kinds of feeding mixtures (Kroulík, 1989). Soybean is used widely and therefore it plays an important role worldwide. Soybean fat-free flour and fat flour is used in feeding mixtures for poultry, pigs and young cattle.

Hygienic safety of foodstuff crucially depends on contamination by microscopic fungi. Besides direct losses caused by their growing (scolding), microscopic fungi also pose risk for human and animal health via production of allergen and toxin (Krysinska-Traczyk & Dutkiewicz, 2000).

Chelkowski (1991) established that the amount of microscopic fungi in fodder is an important indicator of quality and should not be higher than 1.10^5 CFU per kg.

From the samples tested 13 fungal genera were isolated (Tab. 2). The most frequent isolated genera were *Penicillium, Aspergillus, Scopulariopsis, Mucor, Rhizopus* and *Cladosporium*. It is necessary to point out that the genera isolated *Aspergillus, Penicillium. Claviceps* and *Fusarium* are considered to be the most important producers of mycotoxins (Diekman & Green, 1992).

Feedstuff affected by micromycetes of genera *Aspergillus* and *Fusarium* do not often differ from not affected ones. Microscopis fungi have become bioindicators of hygienic suitability of feedstuff, feedstuff and parts of the environment. Attention should be paid not only to their quantity but also to individual species presence in feedstuff (Sweeney & Dobson, 1998).

The isolated and identified species of the *Aspergillus* genus were as follows: *A. candidus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A.* sp. (not identified), *A sydowii, A. versicolor* (Tab. 3). When evaluating fungi presence in various substrates, the species occurrence is as important as their overall quantity. The occurrence of *Aspergillus fumigatus* in poultry feeding mixtures is inadmissible and the occurrence of *Aspergillus flavus* is limited (Tancinová et al., 2001).

Although there is evidence in literature that with a passage of time the number of *Alternaria* in soybean can be reduced to zero after 14 weeks (Jesenská, 1987). We have found that frequency of field fungi was not influenced by storage time. This holds especially for *Alternaria* (Table 2). The frequency of *Alternaria* occurrence in the soybean samples of all farms was not influenced by storage time. At harvest there is a high occurrence of *Alternaria* in soybean that can persist during the whole storage if the grain moisture is lower. However when the grain moisture is higher the typical storage fungi (*Aspergillus, Penicillium*) start to grow up and consequently they have antagonistic effect on the vitality of *Alternaria* and this fungus soon dies (Wilson et al., 2002). Thus the *Alternaria* occurrence can serve as an indicator of recently harvested soybean or good storage conditions (Christensen, 1987).

Acknowledgements

We thank M. Tonková and D. Koštálová for excellent laboratory assistance.

References

1. CHELKOWSKI J. Mycological quality of mixed feeds and ingredients, S 217-227. In: CHELKOWSKI, J. (ed) Cereal Grain, Mycotoxins, Fungi and Quality in Raying and Storage, Elsevier, Amsterdam. 1991.
2. CHRISTENSEN CM. Field and storage fungi, S 211-232. In: BEUCHAT L R. (eds) Food and beverage mycology, Van Nostrand Reinhold, New York. 1987.
3. DIEKMAN M A, GREEN ML. Mycotoxins and reproduction in domestic livestock. J. Anim. Sci. 70: 1615-1627. 1992.
4. HUIS IN'T VELD JHJ. Microbial and biochemical spoilage of foods: an overview. Int. J. Food. Microbiol. 33: 1-18. 1996.
5. JESENSKÁ Z. Mikroskopické huby v pozívatinách a krmivách (Microscopic fungi in the food and forage) . S 319. Alfa, Bratislava, 1987.
6. KROULÍK J. Vyziva a krmivárstvo (Nutrition and feedstuff production). S 201. Príroda, Bratislava, 1989.
7. KUBÁTOVÁ A. Nové druhy toxinogenních penicilií nalezené na potravinách a jejích identifikace. (New species of toxinogenic *Penicillium* found in the foods and their identification) Sb. prednášek: Aktuální problematika mikrobiologie potravin II. Liblice - Byšice: Dum vedeckych pracovníku Akademie ved, S.103-107. 2000.
8. KRYSINSKA-TRACZYK E, DUTKIEWICZ J. *Aspergillus* candidus: a respiratori hazard asssociated with grain dust. Ann. Agric. Environ. Med. 7: 101-109. 2000.
9. LACE, J. Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. J. off Appl. Bact. Symposium Suppl., 11-25.1989.
10. TANCINOVÁ D, KACÁNIOVÁ M, JAVOREKOVÁ S. Natural occurrence of fungi in feeding wheat after harvest and during storage in the agricultural farm facilities. Biologia, 3: 247-250. 2001
11. PITT JI, HOCKING AD. Fungi and food spoilage (2. ed). S 593. London et al. 1997.
12. SAMSON RA, HOEKSTRA ES, FRISVAD JC, FILTENBORG O. Introduction to food-borne fungi. S 322. Vegeningen. 1995.
13. SWEENEY MJ, DOBSON ADW. Mycotoxin production by *Aspergillus, Fusarium* and *Penicillium* species.: Inter. J. Food Microbiology, 43: 141-158. 1998.
14. WILSON DM, MUBATANHEMA W, JURIEVIC Z. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. Adv. Exp. Med. Biol., 504 : 3-17. 2002.