http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (24): 4432-4437, 2007
ISSN 1028-8880

Mycoflora of Maize Harvested from Iran and Imported Maize

[1]Ali Reza Khosravi, [2]Mahdi Mansouri, [3]Ali Reza Bahonar and [1]Hojjatollah Shokri
[1]Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Microbiology, Faculty of Veterinary Medicine, Azad University of Garmsar, Garmsar, Iran
[3]Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Abstract: The natural occurrence of fungal contamination was evaluated in stored maize in three different agro-ecological zones (Iran, Brazil and China). A total of 45 samples were analyzed and 685 fungal isolates were identified. The most frequent isolated fungi from maize originated from Iran, Brazil and China were *Fusarium* sp. (17.3, 17.9 and 37.1%), *Aspergillus* sp. (9.3, 17.4 and 19.7%), *Penicillium* sp. (5.8, 15.2 and 17.6%), *Rhizopus* sp. (2.4, 3.2 and 3.5%), *Mucor* sp. (1.1, 1.6 and 1.3%), *Cladosporium* sp. (1.6, 1.9 and 1.9%), *Alternaria* sp. (1.1, 1.6 and 1.3%), *Geotrichum* sp. (0, 0 and 0.3%), *Acromonium* sp. (0.5, 0.8 and 0%) and *Absidia* sp. (0, 0.8 and 0.5%), respectively. Significant difference was observed between the frequency of fungal isolates of Iranian maize and foreign products ($p<0.0005$). Maize mycoflora profiles showed that *Fusarium verticillioides* and *Aspergillus flavus* prevailed in 30.7 and 13.3% of the samples from China, in 12 and 5.3% of the samples from Iran and 11.7 and 11.5% of the samples from Brazil, respectively. There were significant differences in the frequency of *Fusarium verticillioides* in Chinese maize with other countries products ($p<0.0005$) and that of *Aspergillus flavus* in Iranian maize with other countries ($p<0.002$). The results emphasize that farmers and consumers should be alerted to the danger of fungal contamination in maize.

Key words: Maize, mycoflora, *Fusarium verticillioides*, *Aspergillus flavus*, food control

INTRODUCTION

The increasing worldwide concern about food safety has enhanced interest in fungal contamination and subsequent production of mycotoxins in food products. In this regard, attention is continuously focused on maize (*Zea mays* L.) because it is one of the most important dietary staple foods and feedstuffs in different regions of the world (FAO, 2002). Maize plays an important role in the diet of millions of people due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Asiedu, 1989). Its economical importance is also relevant for its use as feedstuff, mainly in the economically developed countries (Munkvold and Desjardins, 1997). Reports indicate that maize is prone to fungal infection during the pre and post harvest period (Hussein and Brasel, 2001; Abarca *et al*., 2001). Vasanthkumar (1986) demonstrated the infection of maize by field and storage fungi during pre- and post-harvest practices in relation to seed-borne fungal diseases of maize. The contamination of foods and feeds usually reflects the incidence of fungal infection on the original crops such as maize, which is affected by factors including environmental conditions (climate, temperature and humidity), insect infestation and pre- and post-harvest handling (Kacaniova, 2003). In the field as well as in the store, many pests and parasites attack maize. Insects are most often considered as the principal cause of grain losses (Gwinner *et al*., 1996). However, fungi are also second important cause of deterioration and loss of maize (Scudamore and MacDonald, 2000). Kossou and Aho (1993) reported that fungi could cause about 50-80% of damage on farmers maize during the storage period if conditions are favorable for their development. The major genera commonly encountered on maize in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium*. This is a cause of concern because these genera have species capable of producing a wide spectrum of compounds shown to be toxic to man and animals (Orsi *et al*., 2000). Also, its nutritional characteristics expose it to the constant attack of fungi and insect predators (Samson, 1991). *Fusarium verticillioides* (*F. verticillioides*), *F. proliferatum*, *F. oxysporum*, *F. graminearum*, *Aspergillus flavus* (*A. flavus*), *A. parasiticus*, *Cladosporium* sp. and *Penicillium* sp. are the most common seed-borne maize mycoflora, with *F. verticillioides* being the most frequent

Corresponding Author: Dr. Ali Reza Khosravi, Mycology Research Center, Faculty of Veterinary Medicine, Azadi Street, Tehran, Iran Tel: 00982161117151 Fax: 00982166933222

fungus present in maize products (Gonzáles *et al*., 1995; Kedera *et al*., 1999). A major problem related to fungal attack in maize is the production of toxic secondary metabolites, particularly fumonisin, zearalenone and aflatoxin, produced by *F. verticillioides*, *F. graminearum* and *A. flavus*, respectively (Scott and Zummo, 1994). Zearalenone is an estrogenic compound that produces infertility and other reproductive problems in animals (Ngoko *et al*., 2001). Aflatoxin has powerful teratogenic, mutagenic and hepato-carcinogenic effects (Wang *et al*., 2001), while fumonisins are reported to have cancer-promoting activity, in addition to causing several diseases in animals (Bullerman and Draughon, 1994; Rheeder *et al*., 2002). The co-occurrence of fumonisin with aflatoxin B1 (AFB1) is presumed to play an important role in the promotion of carcinogenesis (Ueno, 2000). Both fungal and mycotoxin contamination are currently regarded as the primary concern in the effort to reduce problems in the global trade of agricultural commodities. Hence, there is a great need for more extensive investigations, where maize production and consumption are predominant. Since, the naturally fungal contamination of maize based-foods and feedstuffs from Iran had not been studied to date, the aim of the present study, was to determine mycoflora distribution of Iranian maize and to compare it with imported maize into Iran (Brazilian and Chinese maize).

MATERIALS AND METHODS

Sampling: A total of 45 samples originated from 45 soils, which were suspected to visual contamination, (Iranian, No. = 15; Brazilian, No. = 15; Chinese maize, No. = 15) were collected from different locations of Iran in 2006. The samples were collected in plastic bags, transferred to Mycology Research Center and stored at 4°C until analysis.

Sample culture: One hundred grams from each sample were partially sterilized in a 0.4% sodium hypochlorite solution for 2 min. Subsequently, the supernatant solution was discarded and the sample was rinsed once in distilled water and followed to dry. Twenty-five grains of each sample were homogenized and inoculated into Dichloran Rose-Bengal Chloramphenicol (DRBC, Sigma, St. Louis, USA) agar, 5 grains in a Petri dish. The cultures were then incubated for 5 to 14 days at 25°C in a dark chamber. The colonies were exactly isolated and sub-cultured on slant (PDA) and (SGA) media. *Fusarium* species were isolated, transferred onto Spezieller Nahrstoffarmer agar and incubated at 25°C for 7 days. Final identification of *Fusarium* and *Aspergillus* species was conducted according to Nelson **et al.** (1983) and Raper and Fennell (1965) methods, respectively. The other genera were identified using PDA and SGA media. All chemicals used, unless otherwise stated, were obtained from Merck Company (Darmstadt, Germany).

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

RESULTS

Of the 45 samples examined, which had been collected from Iran, 15 samples and imported foreign samples, 15 from Brazil and 15 from China, 147 (21.5%), 225 (32.8%) and 313 (45.7%) fungal isolates were isolated, respectively. Significant difference was observed between the frequency of fungal isolates of Iranian maize and imported samples ($p<0.0005$). The most frequent isolated fungi from maize originated from Iran, Brazil and China were *F. verticillioides* (12, 11.7 and 30.7%), *F. proliferatum* (3.7, 4.3 and 4.5 %), other *Fusarium* sp. (1.6, 1.9 and 1.9%), *A. flavus* (5.3, 11.5 and 13.3%), *A. ochraceous* (1.6, 2.4 and 2.7%), *A. niger* (1.3, 1.9 and 2.1%), other *Aspergillus* sp. (1.1, 1.6 and 1.6%), *Penicillium* sp. (5.8, 15.2 and 17.6%), *Rhizopus* sp. (2.4, 3.2 and 3.5%), *Mucor* sp. (1.1, 1.6 and 1.3%), *Cladosporium* sp. (1.6, 1.9 and 1.9%), *Alternaria* sp. (1.1, 1.6 and 1.3%), *Geotrichum* sp. (0, 0 and 0.3%), *Acromonium* sp. (0.5, 0.8 and 0%) and *Absidia* sp. (0, 0.8 and 0.5%) (Table 1). Among the *Fusarium* sp., *F. verticillioides* was the most prevalent isolated fungus in Chinese (30.7%), followed by Iranian (12%) and Brazilian (11.73%) maize. There was significant difference in the frequency of *F. verticillioides* in Chinese maize with other countries samples ($p<0.0005$), whereas no significant difference was observed between Iranian and Brazilian maize. Also, significant differences were not observed between other *Fusarium* species with different origins. Overall, *Fusarium* sp. were the most prevalent isolated fungi of maize collected from Iran (17.3%), Brazil (17.9%) and China (37.1%). Mycological analyses showed that *Aspergillus* sp. was the second predominant fungal genus in maize, understudy. *Aspergillus* sp. prevailed in 9.3, 17.3 and 19.7% from Iranian, Brazilian and Chinese samples, respectively. *Aspergillus* flavus was the most frequent species in Chinese (13.3%), followed by Brazilian (11.5%) and Iranian (5.3%) maize. There was only significant difference between Iranian maize with other countries samples ($p<0.002$), whereas significant differences were not observed between other *Aspergillus* sp. with different origins. The incidence of *Penicillium* sp. was about 5.9, 15.2 and 17.6% from

Table 1: The relative frequency of fungal isolates from Iranian, Brazilian and Chinese maize

Fungal isolate	Chinese maize	Brazilian maize	Iranian maize	p-value
Fusarium verticillioides	30.7	11.7	12.0	0.0005
Fusarium proliferatum	4.5	4.3	3.7	NS
Fusarium sp.	1.9	1.9	1.6	NS
Aspergillus flavus	13.3	11.5	5.3	0.002
Aspergillus ochraceous	2.7	2.4	1.6	NS
Aspergillus niger	2.1	1.9	1.3	NS
Aspergillus sp.	1.6	1.6	1.1	NS
Penicillium sp.	17.6	15.2	5.9	0.0005
Rhizopus sp.	3.5	3.2	2.4	NS
Mucor sp.	1.6	1.3	1.1	NS
Cladosporium sp.	1.9	1.9	1.6	NS
Alternaria sp.	1.3	1.6	1.1	NS
Geotrichum sp.	0.3	0.0	0.0	NS
Acromonium sp.	0.0	0.8	0.5	NS
Absidia sp.	0.5	0.8	0.0	NS

NS: Not Significant

Iranian, Brazilian and Chinese samples, respectively. There was significant difference in the frequency of *Penicillium* sp. in Iranian maize with other countries samples ($p<0.0005$), whereas no significant difference was observed between Chinese and Brazilian maize. The results showed that there were not significant differences between the frequency of *Rhizopus* sp., *Mucor* sp., *Cladosporium* sp. and *Alternaria* sp. with different origins.

DISCUSSION

Fungi are worldwide microorganisms and tropical climates stimulate the growth of toxigenic fungi on agricultural products, with consequent risk of mycotoxin contamination (Hell *et al*., 2000). Maize as a cereal crop is grown throughout the world and plays an important role in both human and animal nutrition (Asiedu, 1989). China and Brazil has been ranked as the second and third largest world producer, following the united state. Since, Iran is one of the importers of maize from other countries, especially China and Brazil and regarding to the relative high incidence of mycotoxicosis in animals and to lesser extent in human, fungal contamination was investigated in maize collected from infected sites with different sources. In the present study, the most important isolated fungi were *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. in different samples. Literature reviews showed that the major genera commonly encountered on maize in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium* (Orsi *et al*., 2000; Ghiasian *et al*., 2004). The predominance of *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. in freshly harvested maize grains was also shown by Lillehoj and Zuber (1988) in a work carried out with samples from different countries. In a study conducted by Ghiasian *et al*. (2004) in Iran, *Fusarium* (38.5%), followed by *Aspergillus* (8.7%), *Rhizopus* (4.8%), *Penicillium* (4.5%), *Mucor* sp. (1.1%) and four other fungal genera were noted. *Fusarium verticillioides* was the most prevalent species (83% of *Fusarium* isolates and 52% of the total isolations). Among the *Aspergillus* sp., *A. flavus* was the most widely recovered species and 38% of samples were contaminated with this potentially aflatoxigenic fungus. *Penicillium* sp. were seen in all the samples. Almeida *et al*. (2000) reported that fungal contamination of 66 samples of freshly harvested maize grains collected in different regions of the state of São Paulo (Brazil) were *Fusarium* sp. (80.0%), *Penicillium* sp. (40.0%), *Aspergillus* sp. (23.3%) and *Geotrichum* sp. (23.3%). These species are natural contaminants of cereals worldwide and are mostly found in maize and its derived products (Shephard *et al*., 2000). Gonzales *et al*. (1995) indicated the isolation of around 20 different species, with some of them being potentially toxigenic fungi such as *A. fumigatus*, *A. parasiticus*, *F. verticillioides* and *Monascus rube* from maize. Previous studies revealed that *F. verticillioides*, *F. proliferatum*, *F. oxysporum*, *F. graminearum*, *A. flavus*, *A. parasiticus*, *Cladosporium* sp. and *Penicillium* sp. were the most common seed-borne maize mycoflora, with *F. verticillioides* being the most frequent fungus present in maize (Scott and Zummo, 1994; Kedera *et al*., 1999). As described in this study, species of *Fusarium* were the most prevalent component of maize mycoflora present in all samples. Among them, *F. verticillioides* (12%) and then *F. proliferatum* (3.7%) were the predominant *Fusarium* isolated from Iranian maize and was found at higher quantity than that observed on commercial maize harvested in Brazil (11.7 and 4.3%) and lower quantity than Chinese maize (30.7 and 4.5%). *Fusarium verticillioides* and *F. proliferatum* co-occur worldwide on maize (Leslie *et al*., 1995), probably because they have similar optimum growth conditions and they do not show apparent antagonism when growing together (Logrieco and Moretti, 1995). Doko *et al*. (1995) reported *F. verticillioides* is the most frequently isolated fungus from maize and maize based commodities in France, Spain and Italy. Likewise, Orsi *et al*. (2000) found in Brazil that *F. verticillioides* was the predominant *Fusarium* species on maize. Also, a high occurrence of *F. verticillioides* associated with natural fumonisin contamination has been found in maize in the State of Paraná, which produces 25% of the maize crop in Brazil (Ono *et al*., 2001). However, reports of surveys conducted in some African countries showed it as the most prevalent fungus on maize (Scott and Zummo, 1994; Allah Fadl, 1998). It has been reported that late planting of maize with harvesting in wet conditions favors disease caused by *F. verticillioides* (Abarca *et al*., 2001) and the prevalence of this fungus is considerably increased in seasons with wet weather (Al-Heeti, 1987). The type of maize cultivar and grain characteristics such as color, endosperm type, chemical composition and stage of development may also influence fungal infection and subsequent fumonisin production. Among the Aspergillus isolates, the species identified was *A. flavus*. The presence of *A. flavus* in freshly harvested maize was previously observed (Lillehoj *et al*., 1980; Leoni and Soares, 1994; Machinski *et al*., 2000). In general, it is thought that maize cultivars with upright cobs, tight husks (Emerson and Hunter, 1980), thin grain peri carp (Riley *et al*., 1993) and an increased propensity for grain splitting (Odvody *et al*., 1990) are likely to be more susceptible to fungal infection. Considering a high incidence of fungal contamination of imported maize, it seems that 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 maize in the poor environmental conditions including high moisture and temperature in borderlines and barns in Iran. Maize stored for long-time periods are more vulnerable than freshly harvested maize. Insects and rodents may also be contributed to deteriorating the grains rapidly and increasing maize mycoflora during long-term storage (Hussein and Brasel, 2001). It is necessary to mention that the higher fungal contamination of Chinese maize than Brazilian samples is probably related to higher moisture, probable contamination of maize in origin and applied traditional methods in harvesting, drying and also improper handling grains. Rapid deterioration of grains is a major problem related to fungal contamination in maize, leading to the accumulation of mycotoxins, particularly fumonisin, zearalenone and aflatoxin, produced by *F. verticillioides*, *F. graminearum* and *A. flavus*, respectively (Scott and Zummo, 1994). They may cause acute toxicity or decrease productivity in animals and occasionally caused acute intoxication in humans (Dawson, 1991). Several surveys carried out in many parts of the world have revealed that *F. verticillioides*, *F. proliferatum* are the fumonisin-producing *Fusarium* species most frequently isolated from maize in tropical and subtropical zones (Shephard *et al*., 2000). Also, various reports have shown the presence of AFB1 in 12.3% of the maize samples taken from several Brazilian states (Sabino *et al*., 1989). Although the detection of toxigenic fungi in a substrate does not necessarily indicate that mycotoxins are naturally occurring in the field, it alerts to the potential risk of contamination. Therefore, it is important to keep human and animals exposure to fungal and mycotoxin contaminations as low as possible. In this regard, the lack of proper storage facilities induces fungal contamination and accumulation of mycotoxins during the post-harvest period. Therefore, it is suggested that the proper handling of maize during the post-harvest phase is crucial to preserve grains for longer periods.

ACKNOWLEDGMENT

This study was supported by the Research Council of University of Tehran.

REFERENCES

Abarca, M.L., F. Accensi, M.R. Bragulat and F.J. Cabanes, 2001. Current importance of Ochratoxin A producing *Aspergillus* sp. J. Food Prot., 64: 903-906.

Al-Heeti, A.A., 1987. Pathological, toxicological and biological evaluations of. *Fusarium* species associated with ear rot of maize. Ph.D Thesis, Madison University, Wisconsin.

Allah Fadl, M.E., 1998. Occurrence and toxigenicity of *Fusarium moniliforme* from freshly harvested maize ears with special references to fumonisin production in Egypt. Mycopathology, 140: 99-103.

Almeida, A.P., B. Corrêa, M.A.B. Mallozzi, E. Sawazaki and L.M. Valente Soares, 2000. Mycoflora and aflatoxin/fumonisin production by fungal isolates from freshly harvested corn hybrids. Braz. J. Microbiol., 31: 321-326.

Asiedu, J.J., 1989. Processing Tropical Crops. A Technological Approach. The MacMillan Press, London and Basingstoke.

Bullerman, L.B. and F.A. Draughon, 1994. *Fusarium moniliforme* and fumonisin symposium: Introduction. J. Food Prot., 57: 513.

Dawson, R.J., 1991. A Global View of the Mycotoxin Problem. In: Fungi and Mycotoxins in Stored Products, Champ, B.R., E. Highly, A.D. Hocking and J.I. Pitt (Eds.). ACIAR Proceedings of an International Conference, Bangkok, Thailand, 36: 22-28.

Doko, M.B., S. Rapior, A. Visconti and J.E. Schjoth, 1995. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. J. Agric. Food Chem., 43: 429-434.

Emerson, P.M. and R.B. Hunter, 1980. Response of maize hybrids to artificially inoculated ear mould incited by *Gibberella zeae*. Can. J. Plant Sci., 60: 1463.

FAO., 2002. FAOSTAT Database. Food and Agricultural Organisation, Roma, Italy. URL: http://apps.fao.org/page/collections.

Ghiasian, S.A., P. Kord Bache, S.M. Rezayat, A.H. Maghsood and H. Taherkhani, 2004. Mycoflora of Iranian maize harvested in the main production areas in 2000. Mycopathology, 158: 113-121.

Gonzáles, H.H.L., S.L. Resnik, R.T. Boca and W.F.O. Marasas, 1995. Mycoflora of Argentinian corn harvested in the main production area in 1990. Mycopathology, 130: 29-36.

Gwinner, J., R. Harnisch and O. Müch, 1996. Manuel sur la manutention et la conservation des grains après-récolte. GTZ, Eschborn, Germany, pp: 368. URL:http://www.fao.org/inpho/vlibrary/move_rep/x0298f/x0298F31.htm-34k.

Hell, K., K.F. Cardwell, M. Setamou and F. Schulthess, 2000. Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin. Afr. Entomol., 8: 169-177.

Hussein, S.H. and J.M. Brasel, 2001. Toxicity, metabolism and impact of mycotoxins on humans and animals. Toxicology, 167: 101-134.

Kacaniova, M., 2003. Feeding soybean colonization by microscopic fungi. Trakya Univ. J. Sci., 4: 165-168.

Kedera, C.J., R.D. Plattner and A.E. Desjardins, 1999. Incidence of *Fusarium* sp. and levels of fumonisin B1 in maize in western Kenya. Applied Environ. Microbiol., 6: 41-44.

Kossou, D.K. and N. Aho, 1993. Stockage et Conservation Des Grains Alimentaires Tropicaux: Principes et Pratiques. Les Editions du Flamboyant, Benin, pp: 125.

Leoni, L.A.B. and L.M.V. Soares, 1994. Desenvolvimento de uma metodologia para determinação e confirmação de moniliformina em milho. In: Congresso Latino de Micotoxicologia, 1. Encontro Nacional De Micotoxinas, 8. Rio de Janeiro, pp: 114-115.

Leslie, J.F., C.A.S. Pearson, P.E. Nelson and P.A. Toussoun, 1995. *Fusarium* sp. from corn, sorghum and soybean fields in the central and eastern United States. Phytopathology, 80: 343-350.

Lillehoj, E.B., K.F. Kwolek and E.S. Horner, 1980. Aflatoxin contamination of pré-harvest corn: Role of *Aspergillus flavus* inoculum and insect damage. Cereal Chem., 57: 255-257.

Lillehoj, E.B. and M.S. Zuber, 1988. Distribution of toxin-producing fungi in nature corn kernels from diverse environments. Trop. Sci., 28: 19-24.

Logrieco, A. and A. Moretti, 1995. Occurrence and toxigenicity of *F. proliferatum* from preharvest maize ear rot and associated mycotoxins in Italy. Plant Dis., 79: 727-731.

Machinski, M., L.M.V. Soares, E. Sawasaki, D. Bolonhezi, J.L. Castro and N. Bortoletto, 2000. Incidence of A.atoxins, Ochratoxin A and Zearalenone in Corn Cultivars Plantes in the State of Sao Paulo. In: X International IUPAC Symposium on Mycotoxins and Phycotoxins, Sabino, M., D. Rodriguez-Amaya and B. Correa (Eds.). Guaruj_a, Brazil, pp: 142.

Munkvold, G.P. and A.E. Desjardins, 1997. Fumonisins in maize: Can we reduce their occurrence. Plant Dis., 81: 556-565.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University Park, Pa.

Ngoko, Z., W.F.O. Marasas, J.P. Rheeder, G.S. Shephard, M.J. Wingfield and K.F. Cardwell, 2001. Fungal infection and mycotoxin contamination of maize in the humid forest and the western highlands of Cameroon. Phytoparasitica, 29: 352-360.

Odvody, G.N., J.C. Remmers and N.M. Spencer, 1990. Association of kernel splitting with kernel and ear rots of corn in a commercial hybrid grown in the coastal bend of Texas. Phytopathology, 80: 1045.

Ono, E.Y.S., M.A. Ono and F.Y. Funo, 2001. Evaluation of fumonisins-aflatoxins cooccurrence in Brazilian corn hybrids by ELISA. Food Addit. Contam., 18: 719-729.

Orsi, R.B., B. Corrêa and C.R. Possi, 2000. Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize. J. Stored Prod. Res., 36: 75-87.

Raper, K.B. and D.I. Fennell, 1965. The Genus *Aspergillus*. Baltimore, Md: The Williams and Wilkins Co.

Rheeder, J.P., W.F.O. Marasas and H.F. Vismer, 2002. Production of fumonisin analogs by *Fusarium* sp. Applied Environ. Microbiol., 68: 2101-2105.

Riley, R.T., W.P. Norred and C.W. Bacon, 1993. Fungal toxins in foods: Recent concerns. Ann. Rev. Nutr., 13: 167-189.

Sabino, M., G. Prado, E.I. Inomata, M.O. Pedroso and R.V. Garcia, 1989. Natural occurance of aflatoxin and zearalenone in maize in Brazil. Part II. Food Addit. Contam., 6: 327-331.

Samson, R.A., 1991. Identification of Food-Borne *Penicillium, Aspergillus* and *Fusarium* sp. In: Fungi and Mycotoxins in Stored Products, Champ, B.R., E. Highly, A.D. Hocking, J.I. Pitt (Eds.). 2nd Edn. Proceedings of an International Conference, Bangkok, Thailand.

Scott, G.E. and N. Zummo, 1994. Kernel infection and aflatoxin production in maize by *Aspergillus flavus* relative to inoculation and harvest dates. Plant Dis.,78: 123-125.

Scudamore, K.A. and S.J. MacDonald, 2000. A collaborative study of an HPLC method for determination of ochratoxin A in wheat using immuno-affinity column clean-up. Food Addit. Contam., 15: 401-410.

Shephard, G.S., W.F.O. Marasas and N.L. Leggott, 2000. Natural occurrence of fumonisins in corn from Iran. J. Agric. Food Chem., 48: 1860-1864.

Ueno, Y., 2000. Risk of multiple exposure to natural toxins. Mycotoxins, 50: 13-22.

Vasanthkumar, P., 1986. Studies on some seed-borne fungal diseases of maize in Karnataka. Ph.D Thesis, University of Mysore, India.

Wang, J.S., T. Huang and F. Liang, 2001. Hepatocellular carcinoma and aflatoxin exposure in Zhyqing Village, Fusui County, People's Republic of China. Cancer Epidemiol. Biomark., 10: 143-146.