African Crop Science Conference Proceedings, Vol. 6. 335-339
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ISSN 1023-070X $ 4.00

Occurrence of mycotoxin producing F*usarium* species and other fungi on wheat kernels harvested in selected districts of Kenya

J. W. MUTHOMI & E. W. MUTITU
Department of Crop Protection, University of Nairobi, P. O. Box 30197, Nairobi, Kenya.

Abstract Wheat samples collected from 5 wheat growing districts of Kenya were investigated for contamination by different fungi. Kernels were plated on agar media and the fungi that grew were identified by cultural and morphological characteristics to genus level. *Fusarium* isolates were identified to species level and isolates of *F. graminearum* were tested for mycotoxin production in culture. The major genera of fungi isolated according to decreasing frequency were *Epicoccum* (52.8%), *Alternaria* (34%), *Fusarium* (6%), *Aspergillus* (2.3%) and *Penicillium* (1.8%). The frequently isolated Epicoccum species was identified as *E. purpurascenes*. *Cladosporium* and *Rhizopus* spp. were also isolated at very low frequencies. The most frequently isolated *Fusarium* species were *F. poae* (43%), *F. graminearum* (39%), and *F. avenaceum* (8%). Other *Fusarium* species isolated were *F. equiseti*, *F. oxysporum*, *F. camptoceras* and *F. chlamydosporium.* Most isolates of *F. graminearum* produced mycotoxin deoxynivalenol and zearalenone. The isolated *Fusarium* species are known to cause head blight in wheat resulting in mycotoxin contamination of the grains. The results therefore indicated that head blight is widely distributed at low levels in the wheat growing areas investigated. This inoculum is potentially capable of producing severe infections under optimum weather conditions.

Key words: *Fusarium*, head blight, mycotoxins, wheat.

Introduction

Fusaria can infect wheat during all growth stages but the most susceptible and economically important developmental stage is at flowering. The common *Fusarium* species involved in wheat fusariosis are *F. graminearum (Gibberella zeae)*, *F. culmorum*, *F. crookwellense*, *F. avenaceum (G. avenacea)*, *F. sporotrichioides*, *F. poae*, and *Microdochium nivale* (*F. nivale*) (Parry *et al*. 1995). *Fusarium graminearum* and *F. culmorum* are the most aggressive, causing severe blighting of wheat ears (Stack & McMullen, 1985). *Fusarium* head blight (scab) has recently re-emerged as a devastating disease of wheat and barley throughout the world (Windels, 2000). Undesirable effects of the disease include reduction of grain yield and quality, mycotoxin poisoning in livestock fed with contaminated cereals and mycotoxin carry-over to food products (Chelkowski, 1998). Many of the species responsible for *Fusarium* head blight can also cause wheat seedling blight and brown foot rot (Parry *et al*. 1995). The pathogens survives in old stalks and ears of maize, and on stubble and debris of wheat, barley and other cereals. Other fungi that infect wheat grain in the field include *Alternaria*, *Fusarium*, *Helminthosporium*, *Epicoccum*, *Cladosporium*, *Chaetomium*, *Curvularia*, *Myrothecium*, *Rhizopus* and *Stemphylium* Spp. (Zillinsky, 1983). They infect seed when relative humidity exceeds 90% and seed moisture content exceeds 20%. Most of these fungi are saprophytic but some are pathogenic to seedlings that develop from infected seed. Infected grain has low market value because its products have undesirable colour and odour. In Kenya, little information is available regarding the microflora on wheat grain. This study was therefore carried out with the aim of identifying the fungi contaminating wheat kernels in some selected wheat-growing areas of Kenya.

Materials and methods

Sample collection, isolation and identification of fungi. One kilogram wheat samples were collected at random during harvesting or immediately after harvest from farmers and also from the main markets from the following 5 wheat-growing districts of Kenya: Nakuru (23 samples), Nyandarua (22 samples), Laikipia (24 samples) Meru (20 samples) and Narok (15 samples). Isolation was done within 20 days of collection. Isolation medium was Czapek-Dox-Iprodione-Dicloran agar-CZID (Abildgren *et al*, 1987). Ten surface-sterilized kernels were kernels were evenly placed in each petri dish over the molten agar medium cooled to 45°C such that 100 kernels were plated for each sample. The plates were incubated at room temperature for 7 to 14 days, after which the number of kernels showing fungal infection were recorded. Fungal colonies were sub-cultured on potato dextrose agar (PDA) and identified to genus level following descriptions of Barnet and Hunter (1972) and Zillinsky (1983) based on cultural characteristics and spore morphology.

The relative isolation frequency (Fq) of each genus was calculated as follows (Gonzalez *et al*.1999).

$$Fq\ (\%) = \frac{\text{Number of isolates of a genus}}{\text{Total number of fungi or genus}} \times 100$$

Fusarium colonies growing from the kernels were sub-cultured on (PDA) and Synthetic Nutrient Agar (SNA, Nirenberg, 1981). The cultures were incubated under near UV black light for 14 - 21 days and identified to species level according to Nelson *et al*. (1983). PDA was used for cultural characterization based on rate of growth, presence of aerial mycelium, colour of aerial mycelium and reverse colony colour. Cultures grown on SNA were used for microscopic identification based on spore and conidiophore morphology. Identification features used were macroconidia morphology (size, shape of basal and apical cells), microconidia (present or absent, whether produced in chains or false heads, shape), type of conidiophores and chlamydospores (present or absent, arrangement).

Mycotoxin production: Mycotoxin production in culture was tested on autoclaved, course ground maize (Muthomi *et al*, 2000). Ten grams of the course ground maize in 100 ml Erlenmeyer flasks were moistened with 7.5ml distilled water and autoclaved at 120^{o}C for 20 min. in two consecutive days. After cooling, each flask was inoculated with two 10mm diameter agar disks cut from 7 to 14-day-old cultures and incubated under near U-V black light for 14 days. Culture clumps were broken, dried at 60^{o}C for 24h and ground to fine powder. Five grams of the ground sample was used for mycotoxin extraction with acetonitrile-water (3:1 v/v) and cleaned up as described by Muthomi *et al* (2000, 2002a). Mycotoxins were detected and quantified by HPLC (Muthomi *et al*, 2000). A total of 41 *F. graminearum* isolates were tested.

Results

Epicoccum and *Alternaria* were the most frequently fungal genera, giving a mean isolation frequency of 52.8% and 34%, respectively (Fig. 1). High kernel infection with *Epicoccum* and *Alternari*a was detected in samples collected from all the 5 districts investigated while *Fusarium* was most isolated from samples collected from Meru and Nyandarua (Table 1). *Epicoccum purpurascenes* was the most frequently identified species. *Fusarium* had a mean isolation frequency of 6.4%. Storage fungi like *Aspergillus* and *Penicillium* were isolated at very low frequencies mainly from samples bought at the market.

Of the *Fusarium* species isolated 80 isolates were *F. graminearum*, 89 *F. poae*, 17 *F. avenaceum*, 9 *F. oxysporum*, 3 *F. equiseti* and 8 belonged to other *Fusarium* species including *F. camptoceras* and *F. chlamydosporum*. The over-all relative frequency of the three major *Fusarium* species was 38.8% for *F. graminearum*, 43.2% for *F. poae* and 8.3% for *F. avenaceum*.

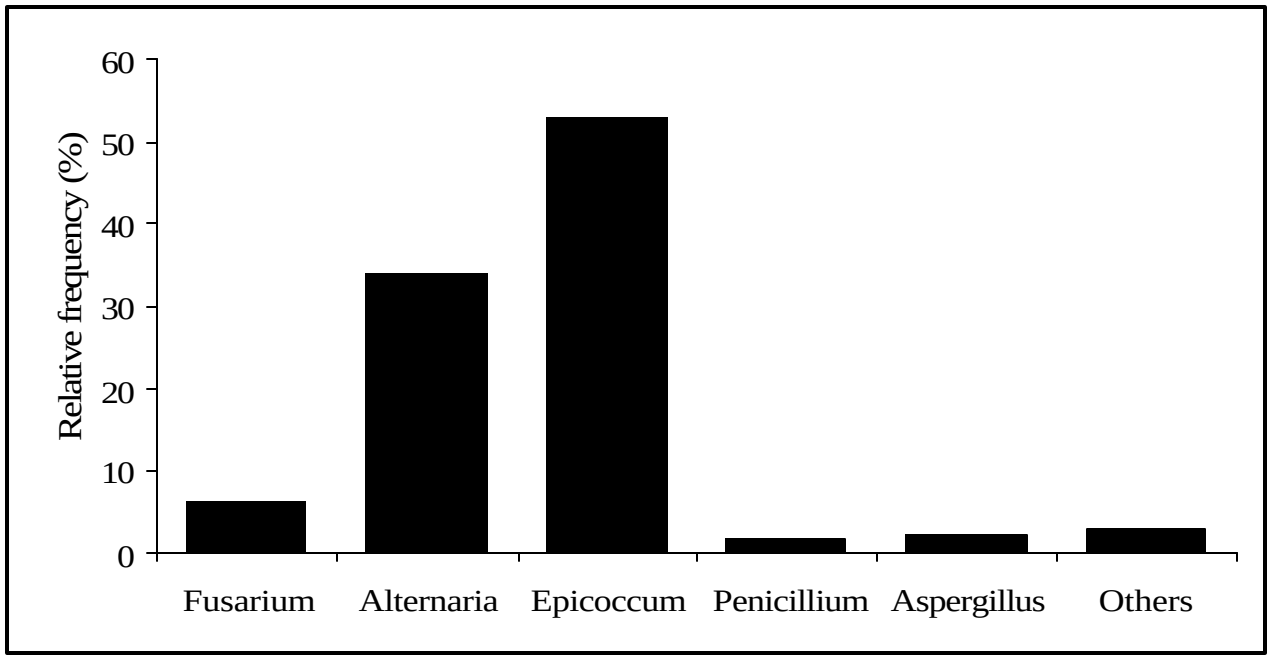
These three species were also widely distributed in the five wheat growing districts (Table 2). *F. graminearum* was most frequently isolated in samples from Nyandarua and Laikipia, while *F. poae* was most frequent in samples from Meru and Nakuru. *F. avenaceum* was most frequent in Meru samples. The *F. graminearum* isolates produced mycotoxins deoxynivalenol ranging from 3.8 to 576µg/g with a mean of 140.7µg/g, zearalenonone 5 to 637 µg/g with a mean of 84µg/g and nivalenol 147 to 338µg/g with a mean 229.9µg/g. All the 41 isolates tested produced zearalenone, 35 produced deoxynivalenol and only 4 produced nivalenol (Fig. 2). There was great variation in mycotoxin production but most isolates produced less than 200µg/g deoxynivalenol and 100µg/g zearalenone.

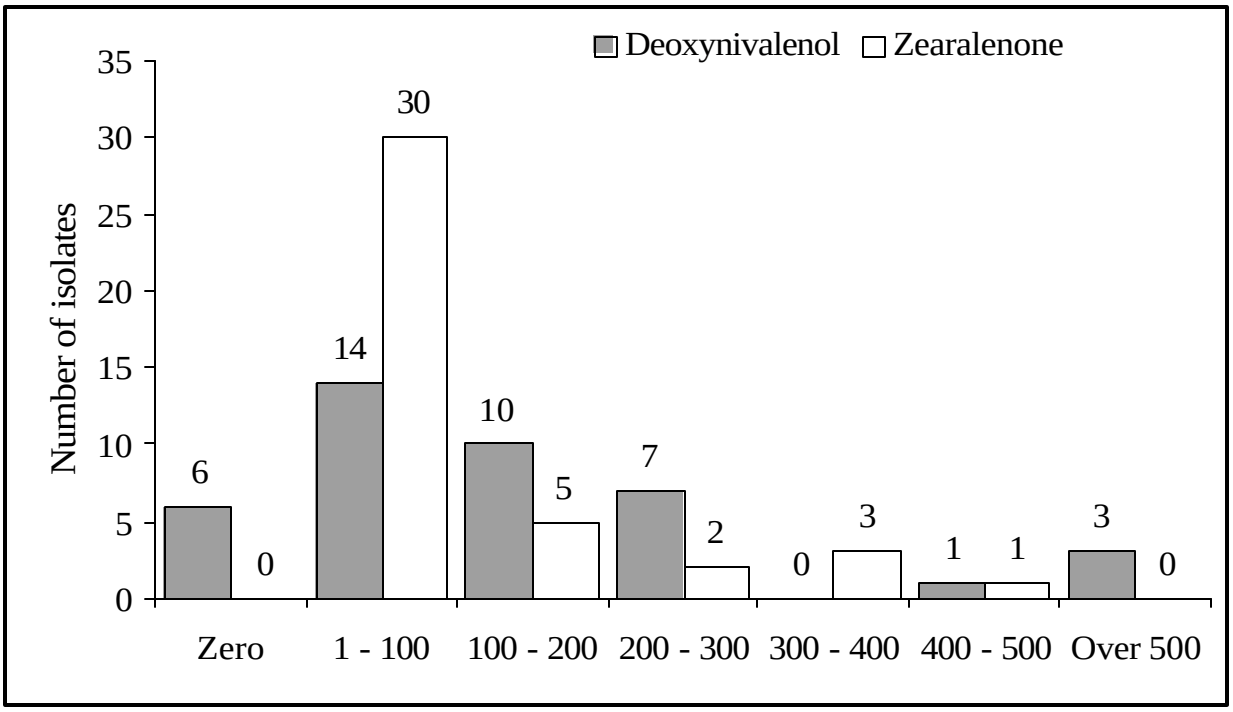
Discussion

Wheat samples were highly contaminated with *Epicoccum* and *Alternaria* spp. but *Fusarium* spp. were detected at low levels. *Epicoccum* and *Alternaria* are mainly saprophytic causing grey or black discoloration of heads and seeds resulting in sooty moulds, black point, or smudge (Zillinsky, 1983). However, some *Alternaria* especially *A. alternata* produce mycotoxins like alternariol, which is a possible food contaminants (Bottalico and Logrieco, 1996). Storage fungi such as *Aspergillus* and *Penicillium* were detected at very low levels, mainly from samples collected from markets. These fungi produce aflatoxin and ochratoxin on stored grain.

The frequently isolated *Fusarium* species were *F. graminearum*, *F. poae* and *F. avenaceum*. These species are pathogenic to wheat ears, causing head blight and mycotoxin contamination of the resulting grain (Parry *et al*. 1995). Majority of the *F. graminearum* isolates investigated produced mycotoxins deoxynivalenol and zearalenone in culture but very few isolates produced nivalenol. Other artificial inoculation experiments showed that the same isolates were virulent on wheat ears resulting in grain contamination with the same mycotoxins (Muthomi, 2001; Muthomi *et al*. 2000b). Furthermore, 15 locally grown wheat varieties were found to be susceptible to *Fusarium* head blight (Muthomi, 2001; Muthomi *et al*, 2002a). Mycotoxin production is a common feature of *F. graminearum* and *F. culmorum* and isolates that produce deoxynivalenol and nivalenol in culture also produce the respective mycotoxins under field conditions (Gang *et al*, 1998; Ichinoe *et al*, 1983; Muthomi *et al*, 2000).

Therefore, *in vitro* mycotoxin studies can be used to predict the type of mycotoxins produced in the field. Pevalence of *Fusarium* species was found to be quite low at 4 - 9% kernel contamination. However, this is not a true reflection of field situation because severely infected and shrivelled kernels, which are very light in weight, are expelled with chaff during combine harvesting (Bai and Shaner, 1994). This not withstanding, the low levels of inoculum detected are potentially capable of producing severe head blight infections and mycotoxin risk given optimum weather conditions. Critical conditions include a wheat crop at


60
50
40
30
20
10
0
Relative frequency (%)
Fusarium
Alternaria
Epicoccum
Penicillium
Aspergillus
Others



flowering to hard milk stage and temperatures higher than 18° C accompanied by more than 5 mm rainfall for at least 24 h (Parry *et al*. 1995). Wheat-maize rotations result in higher head blight infections and their stubble is a good reservoir for *F. graminearum* (Dill-Macky and Jones, 2000; Parry *et al*. 1995).

Acknowledgement

This research was funded by German Academic Exchange Service (DAAD) as part of Ph.D. thesis for the first author.

References

Albildgren, M. P., Lund, F., Thrane, U., & Elmholts, S. 1987. Czapek Dox agar containing Iprodione as a selective medium for the isolation of *Fusarium* species. Lt. Appl. Microbiol. 5: 83 - 86.

Bai, G. & Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Disease 78, 760-766.

Barnett, H. L. & Hunter, B. B. 1972. Illustrated genera of imperfect fungi, 3rd Ed. Burgess Publishing Company, Minnesota. 241 pp.

Bottalico, A. & Logrieco, A. 1998. Toxigenic *Alternaria* species of economic importance. In: Sinha, K. K. and Bhatnagar, D. (Eds.). Mycotoxins in agriculture and food safety. Marcel Dekker, Inc. pp65-108.

Chelkowski, J. 1998. Distributiopn of *Fusarium* species and their mycotoxins in cereal grains. In: Sinha, K. K. and Bhatnagar, D. (Eds.). Mycotoxins in agriculture and food safety. Marcel Dekker, Inc. pp45 - 64.

Dill-Macky, R. & Jones, R. K. 2000. The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. Plant Disease, 84: 71-76.

Gang. G., T. Miedaner, U. Schuhmachar, M. Schollenberger & H. H. Geiger. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness towards winter rye. Phytopathology, 88 879-884.

Gonzalez, H. H. L., Martinez, E. J., Pacin, A. & Resnik, S. L. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum. Mycopathologia 144, 97-102.

Ichinoe, M., H. Kurata, Y. Sigiura & Y. Ueno. 1983. Chemotaxonomy of *Gibberrella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol., 46: 1364-1369.

Muthomi, J. W., A. Schutze, H.-W. Dehne, E. W. Mutitu & E.-C. Oerke 2000. Characterization of *Fusarium culmorum* isolates by mycotoxin production and aggressiveness to winter wheat. Journal of Plant Disease and Protection, 107(2): 113-123.

Muthomi, J. W. 2001. Comparative studies on virulence, genetic variability and mycotoxin production among isolates of *Fusarium* species infecting wheat. Ph.D. Thesis, University of Nairobi.

Muthomi, J. W., E.-C. Oerke, E. W. Mutitu, A. Schade-Schuetze & H.-W. Dehne. 2002a. Susceptibility of Kenyan wheat varieties to head blight, fungal invasion and deoxynivalenol accumulation inoculation with *Fusarium graminearum.* Journal of Phytopathology 150, 30-36.

Muthomi, J. W., E.-C. Oerke, E. W. Mutitu, A. Schade-Schuetze & H.-W. Dehne. 2002b. Variation among *Fusarium* species and isolates infecting wheat ears based on aggressiveness, mycotoxin production and RAPD-PCR analysis. Journal of Plant Disease and Protection 109, (5), 462-477.

Nelson, P. E., Toussoun, T. A. & Marassas, W. F. O.. 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University park. 193pp.

Nirenberg, H. 1981. A simplified method for identifying *Fusarium* species occuring in wheat. Canadian Journal of Botany 59, 1599 - 1609.

Parry, D. W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals- a review. Plant Pathology 44, 207-238.

Stack, R. W. and McMullen, M. P. 1985. Head blighting potential of *Fusarium* species associated with spring wheat heads. Canadian Journal Plant Pathology 7, 79-82.

Windels, C. E. 2000. Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in the Northern Great Plains. Phytopathology 90, 17-21.

Zillinsky, F. J. 1983. Common diseases of small grain cereals: A guide to identification. CIMMYT. 141pp.