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The occurrence of *Fusarium* species and mycotoxins in Kenyan wheat

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ABSTRACT

Freshly harvested wheat grain samples were collected during the 2004 growing season to determine the presence of head blight-causing *Fusarium* species. Fungal contamination was determined by isolation on agar media, while mycotoxin analysis was by direct competitive enzyme-linked immunosorbent assay (ELISA). The wheat grain samples were highly contaminated with fungi, especially *Epicoccum*, *Alternaria* and *Fusarium* species. The mean *Fusarium* infection rate varied from 13% to 18%, with the major head blight-causing species being *Fusarium poae*, *Fusarium graminearum*, *Fusarium equiseti* and *Fusarium avenaceum*. *F. graminearum* isolates were found to be highly virulent (79% disease severity) and significantly reduced kernel weight. Most grain samples were contaminated with mycotoxins, with a mean incidence rate of up to 75% for deoxynivalenol (DON) and 86% for T-2 toxin. Other mycotoxins detected were zearalenone and aflatoxin B1. Co-occurrence of DON, T-2 toxin and zearalenone was found in up to 35% of the samples. The results suggested the presence of *Fusarium* head blight and associated mycotoxins in Kenya. The presence of several mycotoxins, even at such low levels, could pose chronic adverse health effects to human and livestock fed on the contaminated wheat products.
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1. Introduction

Fusarium head blight (FHB) has recently re-emerged as a devastating disease of wheat and other small-grain cereals throughout the world (McMullen et al., 1997; Windels, 2000). The significance of the disease in wheat production is attributed to both yield reduction and mycotoxin contamination of the grain harvested from the infected ears. Yield losses from FHB are due to sterility of the florets and formation of shrivelled, lightweight kernels. The disease is caused by different *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. cerealis*, *F. sporotrichioides* and *Microdochium nivale* (Parry et al., 1995). *F. graminearum* and *F. culmorum* are the most virulent species. All the *Fusarium* species that infect cereals are capable of surviving saprophytically on crop debris (Parry et al., 1995; Jones, 2000). The most favorable conditions for infection are prolonged periods (48–72 h) of high humidity and warm temperatures (25–30 °C). Therefore, a combination of abundant inoculum, prolonged or repeated wet periods during flowering through kernel development and very susceptible cultivars lead to severe yield and quality loss (Obst et al., 1997).

Mycotoxins associated with grain affected by FHB include trichothecenes (deoxynivalenol (DON), nivalenol, T-2 toxin, HT-2 toxin) and zearalenone (Park et al., 1996; Doohan et al., 2003; Llorens et al., 2006). The main trichothecene-producing species are *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. poae* and *Fusarium crookwellense* (Marasas et al., 1984). The occurrence, amount and kind of mycotoxin is dependent on the environment, species of the fungus present, severity of infection and the cultivar or kind of crop (McMullen and Stack, 1999; Mentewab et al., 2000; Doohan et al., 2003).

Management strategies for FHB include crop rotation, seed treatment, planting different cultivars, fungicide application, appropriate use of fertilizers, irrigation, weed control, proper land preparation and timely harvesting (McMullen and Stack, 1999; Mathies and Buchenauer, 2000; Haidukowski et al., 2004). Use of fungicides in the management of FHB has been at most 77% and 89% effective in the reduction of disease severity and mycotoxin content, respectively (Haidukowski et al., 2004). Adjusting the combine to blow out the small, shrivelled kernels can help reduce mycotoxin levels. Currently there are no wheat cultivars with complete resistance to FHB, although some cultivars have significant levels of partial resistance that limit yield loss and mycotoxin accumulation (Pereyra and Dill-Macky, 2004; Wisniewska and Kowalczyk, 2005).

Harvested wheat grain from Kenya has been previously shown to be contaminated with mycotoxin-producing *Fusarium* species (Muthomi and Mutitu, 2003). However, mycotoxin levels of the naturally infected wheat grain have not been determined. Therefore, this study was carried out to determine the level of *Fusarium*

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contamination and associated mycotoxins in freshly harvested wheat from Nakuru and Nyandarua districts of Kenya.

2. Materials and methods

2.1. Survey and mycological analysis

A survey was carried out during the 2004 harvesting season in the wheat-growing districts of Nakuru and Nyandarua, Kenya. Five agroecological zones in Nakuru and four agroecological zones in Nyandarua were selected. The agroecological zones selected were upper highland 2 (UH2—very long cropping season), upper highland 3 (UH3—long cropping season), upper highland 4 (UH4—low rainfall and frost), lower highland 2 (LH2—long cropping season), lower highland 3 (LH3—long cropping season), lower highland 4 (LH4—short to medium cropping season) and upper midland 4 (UM4—medium cropping season) (Jaetzold and Schmidt, 1983). In each agroecological zone, 10 farms were randomly selected, giving a total of 89 farms. Wheat grain samples (1–2 kg per farmer) were collected for mycological and toxin analysis. The samples were stored at 4 °C until analyzed. Each sample was thoroughly mixed and a 100 g sub-sample taken randomly for mycological analysis. The seeds were surface sterilized in 5% sodium hypochlorite containing four drops of Tween 20 for 3 min, and then rinsed three times with distilled water. The kernels were plated on petri plates containing low strength potato dextrose agar (PDA) amended with mineral salts (0.005 g $CuSO_4 \cdot 5H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$) and antimicrobial agents (0.05 g chloramphenicol, 50 mg penicillin, 50 mg tetracycline, 50 mg streptomycin) to suppress growth of fast-growing fungi and bacteria (Muthomi, 2001). A total of 100 seeds were plated per sample. The plates were incubated at room temperature 20 ± 5 °C for 7–14 d, after which kernels showing fungal infection were recorded, and the different fungal colony types determined. The fungal genera growing on the kernels were identified based on cultural and morphological characteristics. The *Fusarium* isolates were identified to species level based on synoptic keys by Nelson et al. (1983). *Fusarium* colonies were sub-cultured on both synthetic nutrient agar (Nirenberg, 1981) and PDA. The cultures were incubated under near-UV light for 14–21 d to induce sporulation.

2.2. Mycotoxin analysis

Mycotoxin content in the wheat grain was determined by direct competitive enzyme-linked immunosorbent assay (ELISA) (Association of Official Analytical Chemists (A.O.A.C), 1995). The antibodies were supplied by the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. A total of 80 samples were analyzed for DON, T-2 toxin, zearalenone, and 41 samples for aflatoxin B1. Each sample was homogenized and 100 g ground to a fine powder. Five grams of the ground sample was extracted with 25 ml of methanol:water (50:50v/v) for aflatoxin, zearalenone and DON and 70:20 (v/v) for T-2 toxin. The extract was de-fatted with 10 ml hexane, and 4 ml of the methanolic layer was diluted to 10% using phosphate buffer solution. For T-2 toxin, the methanolic extract was diluted with an equal volume of distilled water. The 96-well microtitre polystyrene (Maxisorp®, Nunc, Denmark) plates were coated with 100 μl of anti-aflatoxin antiserum K147 (Gathumbi et al., 2001) for aflatoxin B1, anti-DON antiserum DON143/16 (Usleber et al., 1992) for DON and anti-serum ZEA A37 (Usleber et al., 1992) for zearalenone in bicarbonate buffer (pH 9.6) per well. For T-2 toxin, a commercial kit (Ridascreen, r-Biopharm, Germany) was used and the ELISA procedure performed following the manufacturer's recommendations. Absorbance was determined using the spectrophotometer Elisa reader (Uniskan II, Finland) at 450 nm. A calibration curve for the standards for each toxin dilution was plotted using $\log_{10}$ of standards concentration against the percentage inhibition of the standards.

2.3. Virulence of Fusarium species

Twenty isolates of different *Fusarium* species isolated from wheat kernels were inoculated onto spikes of 'Mbuni', a highly susceptible wheat cultivar (Muthomi et al., 2002) under greenhouse conditions. Each isolate was cultured separately at room temperature (22 ± 5 °C) on mung bean medium (Bai and Shaner, 1996) for 14 d. Conidial suspensions of each isolate was harvested and adjusted to 5×10^5 conidia ml^{-1} by counting the conidia using a hemocytometer and diluting them to the required concentration with sterile distilled water. Three drops (0.01%) of Tween 20 were added to ensure uniform conidia dispersion. Wheat ears were inoculated at 50% flowering (GS65, Zadoks et al., 1974) by spraying with a hand sprayer, exposing all spikelets to the inoculum. Controls were treated similarly with distilled water. After inoculation, the ears were incubated under polythene bags for 48 h to ensure high relative humidity for optimal infection. Each isolate was inoculated separately and replicated four times. Head blight severity was visually assessed using a 1–9 scale (Miedaner et al., 1996) as the proportion of bleached spikelets after every 5 d on 10 average sized ears per replicate until physiological maturity (GS92). Mean head blight severity and the area under disease progress curve (AUDPC; Shaner and Finney, 1977) were calculated from the disease severity data. The ears from each replicate of each isolate were harvested separately and threshed to determine grain weight.

2.4. Data analysis

Data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat (Lawes Agricultural Trust Rothamsted Experimental Station, 1998, version 8). Where a treatment effect was significant, pair-wise treatment mean differences were determined by Tukey least significant difference test at 95% confidence limit.

3. Results

The major fungal genera isolated from wheat grain samples were *Epicoccum*, *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* (Table 1(a) and (b)). The mean kernel infection rate was 98.4%, but the level of contamination varied in different agro-ecological zones. The highest total *Fusarium* infection was observed in Nyandarua district where rainfall was higher and better distributed than in Nakuru district (Table 2). Agro-ecological zone LH4 had the highest *Fusarium* infection rates. However, agro-ecological zones with high *Epicoccum* and *Alternaria* infection rates had lower levels of *Fusarium* in both districts. *Fusarium* species isolated were *F. poae*, *F. chlamydosporum*, *F. oxysporum*, *F. graminearum*, *F. equiseti*, *F. verticillioides*, *F. avenaceum*, *F. semitectum*, *F. cerealis*, *F. lateratium*, *F. sporotrichioides*, *F. scirpi*, *F. sambucinum* and *F. solani* (Table 3). *Fusarium poae*, *F. chlamydosporum* and *F. oxysporum* were the most prevalent *Fusarium* spp. in all the agro-ecological zones, while *F. graminearum* was isolated in six out of the nine zones. The least prevalent species were *F. semitectum*, *F. scirpi*, *F. solani* and *F. sporotrichioides*.

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