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Occurrence of *Fusarium* Head Blight of Wheat and Associated Mycotoxins in Narok and Nakuru Counties, Kenya

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Abstract *Fusarium* head blight (FHB) is an important disease of small grain cereals. This study assessed the incidence and severity of FHB of wheat at hard dough stage, and levels of deoxynivalenol and T2-toxin at harvest by direct competitive enzyme linked immuno-sorbent assay. Wheat ears were randomly sampled from 51 farms in Narok County and 51 farms in Nakuru County at hard dough stage while wheat kernels were sampled at harvest. Prevalence of FHB in both Counties was 100%. The mean incidence of FHB was 28.4% and 20.5% in Narok and Nakuru Counties, respectively with 16.9% and 11.7% corresponding severity. Over 14 *Fusarium* spp. were isolated from wheat ears and kernels with *F. avenaceum*, *F. poae* and *F. graminearum* being isolated in the highest incidence. Levels of DON in the kernels ranged from below limit of detection (<LOD) to 623 µg/kg while the concentration of T-2 toxin ranged from <LOD to 69 µg/kg. The levels of DON and T2-toxin in wheat kernels in the two Counties were within the limits set by the European Commission and the United States Food and Drug Administration. The relatively low incidence and severity of FHB correlated with the low levels of DON and T-2 toxin in harvested wheat grains. There is however need to continuously monitor occurrence of FHB and toxin levels in wheat which varies among seasons due to variability in climatic conditions.

Keywords: *Fusarium Head Blight, Deoxynivalenol, T2-Toxin, Wheat*

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1. Introduction

Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal grain worldwide [1], providing approximately 20% of global calories to humans. Narok County is one of the leading wheat growing regions in Kenya contributing approximately 40% of the national production [2]. On the other hand, Nakuru County is Kenya's traditional wheat production region as well as the centre of wheat research in the country where the National Plant Breeding Station - that focuses on research in smallholder wheat technologies - is located [3]. However, wheat production in Kenya is constrained by several factors including diseases, insect pests, variable climatic conditions, inadequate land, and competition from other enterprises like maize, coffee and tea [4].

Infection of wheat by *Fusarium* spp. causes *Fusarium* head blight (FHB), a disease caused by multiple soil borne and residue borne *Fusarium* spp. [5,6,7]. *Fusarium* head blight is a pre-harvest disease although *Fusarium* spp. can grow post-harvest if wet grains are not dried effectively and quickly [8]. In the field, the disease is characterized by premature bleaching of the infected spikelets and formation of orange spore bearing sporodochia at the base of the glumes [9]. Previous studies in Kenya indicate that the prevalence of FHB varied from trace to 100% with yield losses varying correspondingly [5,10]. *Fusarium* head blight results in quantitative reduction in grain yield due to reduced seed weight, reduced kernel set and shriveled kernels that are chalky white [11,12]. In addition, the disease affects the quality of the grains through contamination with mycotoxins [13,14].

The major *Fusarium* spp. associated with FHB worldwide include: *F. graminearum*, *F. poae*, *F. avenaceaum* and *F. culmorum* [8]. However, *F. graminearum*, *F. poae*, *F. avenaceaum*, *F. chlamydosporum* and *F. culmorum* are the major *Fusarium* spp. associated with the disease in Africa [5,10,15,16]. In Kenya, other *Fusarium* spp. commonly associated with FHB include: *F. equiseti*, *F. scirpi*, *F. tricinctum* and *F. oxysporum* [5,10,17]. These *Fusarium* spp. exist in a complex with each other and with other fungal species although the predominance of individual species vary [5,8]. The *Fusarium* spp. that predominate in a region are influenced by environmental and agronomic

factors [6,18] with *F. graminearum* predominating in hotter regions while *F. culmorum* and *F. poae* are the predominant species in cooler regions [8,14].

Fusarium graminearum produces sexual spores called ascospores that can be deposited on wheat spikes at flowering [19]. On the other hand, *F. culmorum* is a soil inhabiting fungus that is a competitive saprophyte and a facultative parasite [20] while *F. avenaceum* is a soil saprophyte whose pathogenicity is generally lower than that of *F. culmorum* and *F. graminearum* [20]. *Fusarium poae* which produces asexual spores that are dispersed by wind and by rain splash to wheat heads [19,21] is a relatively weak pathogen compared to *F. graminearum*, *F. culmorum* and *F. avenaceum* but it produces a diverse number of mycotoxins [20]. *Fusarium chlamydosporum* is commonly found in arid and semi-arid regions in the soil and is a saprophyte on a variety of substrates [20] and has been reported as one of the important causes of FHB in Kenya contaminating wheat mainly with moniliformin [5]. *Fusarium tricinctum*, which is less commonly associated with FHB, grows in highlands where temperatures are low and humidity high [16]. Most *Fusarium* spp. produce inocula, grow best and are most pathogenic to wheat heads at warm temperatures and humid climate [6,22]. Extended periods of greater than 90% relative humidity and 15-30 °C temperature create suitable conditions for infection [23]. Continued wet conditions after flowering may increase the potential of the spores to be blown by wind or rain-splashed to nearby ears [14,24].

The major *Fusarium* mycotoxins contaminating wheat grains include deoxynivalenol (DON), nivalenol (NIV), 3 and 15 – acetyl DON and zearalenone produced by *F. graminearum* and *F. culmorum*; T-2 toxin, HT-2 toxin and nivalenol produced by *F. poae*, *F. sporotrichioides* and *F. langsethiae*; and moniliformin and beauvericin produced by *F. avenaceum* [7,8]. Mycotoxins have been associated with chronic and acute human diseases [25,26] with DON being the most economically important *Fusarium* toxin [6]. Ingestion of DON has high emetic effects, disrupts protein function by inhibiting protein synthesis, results in disorders in lipid metabolism, renal filtration disruption and renal cell DNA methylation and rhabdomycolysis [27,28]. T-2 toxin inhibits protein synthesis and causes secondary disruption of DNA and RNA synthesis [29]. The toxin affects the actively dividing cells lining the gastrointestinal tract, skin, lymphoid and erythroid cells. T-2 toxin can also decrease levels of antibody, immunoglobulins and certain other humoral factors. Observable effects associated with T2-toxin include weight loss, poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased animal productivity [30].

Occurrence of FHB and associated mycotoxins varies among seasons hence the need for continuous monitoring and surveillance for the disease and associated toxins. The objectives of this study was therefore to assess the occurrence of FHB of wheat in the field and contamination of wheat grains with DON and T-2 toxin in Narok and Nakuru Counties of Kenya during the 2013 cropping season. In addition, the diversity of fungal pathogens associated with FHB complex of wheat was determined.

2. Materials and Methods

2.1. Field Sampling and FHB Assessment

Field sampling was carried out in Narok and Nakuru Counties, Kenya at hard dough stage (GS 65-69) and at harvest (GS 92) between June and October 2013 in Narok County and between August and December 2013 in Nakuru County. Wheat ears were sampled at hard dough stage while kernels were sampled at harvest. In Narok County, sampling was done from 51 wheat farms in three agro-ecological zones (AEZ): 15 farms in lower highland 3 (LH3), 18 in lower highland 2 (LH2) and 18 in upper highland 3 (UH3). In Nakuru County, sampling was done from 51 wheat farms in four AEZ; 17 farms in upper highland three (UH3), 11 farms in upper highland two (UH2), 13 farms in upper highland one (UH1) and 10 farms in lower highland three (LH3) (Table 1). Small scale (<8Ha), medium scale (8 – 20Ha) and large scale (>20Ha) farms were systematically sampled in each AEZ by selecting the fifth wheat growing farm in a transect. Distribution of the farms among the different size categories was: 65% small scale, 20% medium scale and 15% large scale. A minimum distance of 200m was observed between individual wheat farms where sampling was done.

Table 1. Characteristics of different wheat growing agro-ecological zones in Narok and Nakuru Counties

AEZ	County	Altitude (m asl)	Annual average rainfall (mm)
LH3	Narok, Nakuru	1850 - 2150	850 – 1100
LH2	Narok	1980 - 2280	1000 – 1300
UH3	Narok, Nakuru	2150 - 2370	1000 – 1200
UH2	Nakuru	2280 - 2970	1100 -1800
UH1	Nakuru	2310 - 2580	800 – 1100

Source: Ministry of Agriculture and GTZ (2007); Ministry of Agriculture, 1987.

Incidence of FHB was determined as the number of blighted heads over the total number of heads within five, $1m^2$ randomly selected quadrants in each farm. Severity of FHB was determined as the proportion of bleached spikelets within five, $1m^2$ randomly selected quadrants in each farm on a scale of 0 – 9 [31]: (1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%). At hard dough stage, at least 20 wheat ears were randomly sampled from different locations in each farm for mycological analysis. At harvest, approximately 1 kg of freshly harvested wheat kernels were sampled from each farm and placed in Kraft bags for mycological and mycotoxin analysis from the same farms where the ears had been sampled. The kernel samples were equally sub-divided into two sub-samples of about 500 g each, one for mycological and the other for mycotoxin analysis and stored at 4 °C until analyzed.

2.2. Isolation and Identification of *Fusarium* spp. from Wheat Ears and Kernels

The wheat ears from each farm were cut into small pieces (≈0.5 cm long) using a sterile scalpel. Sub-samples of harvested kernels were taken after thoroughly mixing the samples. The wheat ears and kernels were surface sterilized in 1.3% sodium hypochlorite and subsequently rinsed three times for two minutes in sterile distilled water. Five pieces of wheat ears and five kernels were plated on each Petri plate on low strength potato dextrose agar (PDA) amended with minerals and antibiotics [32]: (PDA 17g, KH_2PO_4 1.0g, KNO_3 1.0g, $MgSO_4$ 0.5g, Agar 10g) and replicated three times. The plates were incubated for 5-7 days at 25°C under 12 h day light and 12 h darkness cycles. Counts of the total number of infected spikelets and kernels per Petri plate were made.

Fusarium colonies were sub-cultured on PDA and synthetic nutrient agar (SNA) [33]: (KH_2PO_4 1.0g, KNO_3 1.0g, $MgSO_4$ 0.5g, KCl 0.5g, Glucose 0.2g, Agar 20g). *Fusarium* cultures on SNA were incubated at near UV-light at 25°C for 10-14 days to facilitate sporulation while those on PDA were incubated at 25°C for 7-10 days. *Fusarium* pathogens were identified to species level using manuals by [34] and [21]. *Fusarium* cultures on PDA were used for cultural characterization while cultures on SNA were used for microscopic identification. Features used for microscopic identification included morphology of macroconidia and microconidia, type of conidiophores, phiallides and chlamydospores. Other fungal genera were sub-cultured on PDA and identified based on cultural and morphological features like mycelia color, spore shape, pigmentation, septation and sporephore characteristics using the pictorial Atlas of soil and seed fungi [35].

2.3. Determination of Deoxynivalenol and T2-toxin Levels in Wheat Kernels

Approximately 500 g sub-sample of wheat kernels from the 1 kg sample from each farm was ground to fine powder using a grinding mill (Bunn Coffee Mill, Bunn-o-matic Corporation, Springfield, Illinois, USA). Each ground sample was thoroughly mixed and a 5 g sub-sample extracted with 25 ml distilled water for deoxynivalenol and 25 ml methanol/distilled water (70:30 v/v) for T-2 toxin. The extracts were stirred on a magnetic stirrer for 3 and 10 minutes for DON and T-2 toxin, respectively. The extracts were then centrifuged at 350 revolutions per minute for 10 minutes. Samples were diluted with an equal volume of sample dilution buffer provided in the Ridascreen® DON (Art. No. : R5906) and Ridascreen® T-2 toxin (Art. No. : R3801) kits.

Levels of DON and T-2 toxin were determined by direct competitive Enzyme Linked Immuno-Sorbent Assay (ELISA) (R-Biopharm AG, Darmstadt, Germany) [36,37] following the manufacturer's instructions. The limits of detection for the DON and T-2 toxin kits were 18.5 and 3.5 µg/kg, respectively. Absorbance was determined using the spectrophotometer ELISA reader (RIDA®SOFT Win) at 450nm wavelength. A calibration curve for the standards for each toxin was plotted using $\log_{10}$ of standards concentration against the percentage inhibition of the standards and used to determine concentration of the toxins in the samples in parts per billion.

2.4. Data Analysis

Data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of GENSTAT version 15 and the mean differences compared using Fisher's Protected LSD at 5% probability level. Percentage data that were not normally distributed were transformed using arcsine $\sqrt{p}/100$ while other skewed data were transformed to $\log_{10}$ for data analysis and separation of means.

3. Results

3.1. Prevalence, Incidence and Severity of *Fusarium* Head Blight of Wheat

Fusarium head blight was observed in all the farms sampled but the incidence and severity of the disease varied between the two Counties, among farms and agro-ecological zones (Table 2). The highest FHB incidence and severity in Narok County were recorded in UH3 while the lowest were recorded in LH3. In Nakuru County, the highest and the lowest incidence and severity were recorded in LH3 and UH2, respectively. There was no significant ($p \geq 0.05$) difference in FHB severity among the three AEZ in Narok County but incidence significantly ($p \leq 0.05$) varied among the AEZ. However, the disease incidence and severity were not significantly ($p \geq 0.05$) different among the AEZ in Nakuru County.

Table 2. Prevalence, Incidence and Severity of FHB of Wheat in Different Agro-Ecological Zones in Narok and Nakuru Counties, Kenya

County	AEZ	Prevalence (%)	Incidence (%)	Severity [a]
Narok (n = 51)	LH3	100	20.7b	3.9
	LH2	100	25.1ab	4.2
	UH3	100	39.4a	3.8
	Mean	100	28.4	4.0
	LSD ($P \leq 0.05$)		17.2	1.3
Nakuru (n = 51)	UH1	100	11.7a	3.5
	UH2	100	10.6a	3.0
	UH3	100	11.8a	4.0
	LH3	100	12.5a	4.0
	Mean	100	11.7	3.6
	LSD ($p \leq 0.05$)		6.1	2.2

[a] Severity score based on scale by Miedaner et al. (1996): 1 = no symptoms, 2 = <5%, 3 = 5-15%, 4 = 16-25%, 5 = 25-44%, 6 = 46-65%, 7 = 66-85%, 8 = 86-95%, 9 = 96-100%. LH3 - lower highland 3; LH2 - lower highland 2; UH1 – upper highland 1; UH2 – upper highland 2; UH3 - upper highland 3. Means followed by the same letter within the column on FHB incidence are not significantly different ($p \leq 0.05$).

3.2. Incidence of Fungal Pathogens in Wheat Ears at Hard Dough Stage

The major fungal pathogens isolated from wheat ears were: *Epicoccum* spp., *Alternaria* spp., *Chaetomium* spp. and *Fusarium* spp. in Narok County (Table 3); and *Epicoccum*, *Fusarium*, *Alternaria*, and *Aspergillus* in Nakuru County (Table 4). In Narok County, *Epiccocum* spp. and *Alternaria* spp. were the most prevalent with mean incidence of 48.7 and 23.1%, respectively. *Penicillium* spp., *Cladosporium* spp., *Helminthosporium* spp. and *Trichoderma* spp. were isolated in low frequency (<1.0%) in Narok County. In Nakuru County, *Epicoccum* and *Fusarium* spp. were the most prevalent with mean incidence of 83.1 and 25.5%, respectively. *Epicoccum* spp. were the most prevalent in all the agro-ecological zones in both Counties while the average isolation frequency of *Fusarium* spp. was higher in Nakuru than in Narok County. Incidence of the fungal pathogens in wheat ears significantly ($p \leq 0.05$) varied among the agro-ecological zones in both Counties. There was a negative correlation between the incidence of *Fusarium* and *Epicoccum* spp. and *Fusarium* and *Alternaria* spp. in both Counties.

Table 3. Incidence (%) of Fungal Genera in Wheat Ears Sampled at Hard Dough Stage in Three Agro-Ecological Zones of Narok County

AEZ	Epi	Alt	Chaet	Fus	Asp	Macro	Ulo	Stemp	Rhizo	Pyt	Others[a]
LH3	69.3 a	42.7 a	2.7 b	5.3 a	0.0 a	0.0 a	1.8 A	0.0 b	0.0 b	0.0 a	4.9 a
LH2	39.6 b	18.9 b	30.4 a	8.9 a	11.1 a	8.9 a	4.8 a	5.6 a	0.4 b	0.7 a	8.1 a
UH3	37.0 b	7.8 c	28.9 a	11.1 a	11.1 a	3.7 a	3.0 a	2.6 b	5.6 a	2.6 a	7.8 a
Mean	48.7	23.1	20.6	8.4	7.4	4.2	3.2	2.7	2.0	1.1	6.9
LSD ($p \leq 0.05$)	11.8	7.3	8.0	6.9	11.3	9.3	6.7	3.5	2.7	3.1	7.1
CV (%)	12.1	15.9	19.4	41.1	76.5	111.6	105.8	64.0	67.6	137.4	50.9

Epi – *Epicoccum*, Alt – *Alternaria*, Chaet – *Chaetomium*, Fus – *Fusarium*, Asp – *Aspergillus*, Macro – *Macrophomina*, Ulo – *Ulocladium*, Stemp – *Stemphilium*, Rhiz – *Rhizoctonia*, Pyt – *Pythium*. LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3; n = 51.
[a] – Fungal pathogens isolated in low frequency and unidentified fungi. Means followed by different letters within columns are significantly different ($p \leq 0.05$).

Table 4. Incidence (%) of Fungal Genera in Wheat Ears Sampled at Hard Dough Stage in Three Agro-Ecological Zones of Nakuru County

AEZ	*Epicoccum*	*Fusarium*	*Alternaria*	*Aspergillus*
UH1	86.7a	28.2b	10.3ab	2.1bc
UH2	91.5a	15.2c	5.7b	0.0c
UH3	69.0b	45.1a	14.1a	5.5ab
LH3	85.3a	13.4c	12.0a	6.7a
Mean	83.1	25.5	10.5	3.6
LSD ($p \leq 0.05$)	9.8	12.1	5.9	3.5
CV (%)	0.5	6.4	24.6	33.7

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3; UH2 – upper highland 2; UH1 – upper highland 1, n = 51.
Means followed by different letters within columns are significantly different ($p \leq 0.05$).

3.3. Incidence of Fungal Pathogens in Wheat Kernels at Harvest

The fungal pathogens isolated in high incidence from wheat kernels sampled at harvest were: *Epicoccum* spp. and *Alternaria* spp. in Narok County (Table 5); and *Epicoccum* and *Fusarium* spp. in Nakuru County (Table 6). There was greater fungal diversity in kernels sampled from Narok than in Nakuru County. The mean incidence of *Fusarium* spp. was higher in kernels sampled from Nakuru than in Narok County. Incidence of the fungal pathogens was significantly ($p \leq 0.05$) different among the agro-ecological zones. There was a negative correlation between the incidence of *Epicoccum* and *Fusarium* spp. and *Fusarium* and *Alternaria* spp. in both Counties.

Table 5. Incidence (%) of Fungal Genera in Wheat Kernels Sampled at Harvest from Different AEZs of Narok County

AEZ	Epi	Alt	Stemp	Asp	Pen	Chaet	Fus	Macro	Ulo	Helm	Tri	Others[a]
LH3	34.7 b	36.4 a	16.0 a	7.1 b	8.4 a	7.1 a	3.1 a	3.1 A	2.2 a	0.4 a	4.4 a	3.6 a
LH2	36.3 b	41.1 a	14.1 a	11.5 ab	6.7 a	5.6 a	3.7 a	3.3 A	0.7 a	0.4 a	0.4 a	1.5 a
UH3	50.7 a	33.7 a	11.5 a	14.8 a	4.1 a	8.5 a	5.2 a	4.1 A	1.9 a	3.0 a	0.0 a	1.1 a
Mean	40.6	37.1	13.9	11.1	6.4	7.1	4.0	3.5	1.6	1.3	1.6	2.0
LSD ($p \leq 0.05$)	9.6	12.2	8.0	7.2	9.5	6.3	7.1	6.1	2.7	2.9	8.9	2.8
CV (%)	11.8	16.4	28.9	32.5	74.3	44.3	88.3	86.7	85.6	115.6	277.9	76.8

Epi – Epicoccum, Alt – Alternaria, Stemp – Stemphilium, Asp – Aspergillus, Pen – Penicillium, Chaet – Chaetomium, Fus – Fusarium, Macro – Macrophomina, Ulo – Ulocladium, Helm – Helminthosporium, Tri - Trichoderma
[a] – unidentified fungal pathogens and fungi isolated in low frequencies (< 1%). LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3; n = 51. Means followed by different letters within columns are significantly different ($p \leq 0.05$).

Table 6. Incidence (%) of Fungal Genera in Wheat Kernels Sampled at Harvest from Different AEZs of Nakuru County

AEZ	*Epicoccum*	*Fusarium*	*Alternaria*	*Aspergillus*	*Penicillium*
UH1	91.2a	3.8b	2.7c	0.8b	0.0b
UH2	87.7a	3.2b	1.8c	0.5b	0.0b
UH3	66.8b	20.0a	6.2b	8.8a	2.7a
LH3	60.5b	16.5a	11.9a	3.0b	1.5ab
Mean	76.5	10.9	5.7	3.3	1.0
LSD ($p \leq 0.05$)	8.2	5.9	3.3	4.4	1.8
CV (%)	2.2	4.7	11.3	26.6	30.8

LH3 - lower highland 3; LH2 - lower highland 2; UH3 - upper highland 3; UH2 – upper highland 2; UH1 – upper highland 1, n = 51.
Means followed by different letters within columns are significantly different ($p \leq 0.05$).

3.4. Incidence of *Fusarium* spp. in Wheat Ears and Kernels

More than ten *Fusarium* spp. were isolated from the wheat ears in the two Counties (Figure 1). However, the diversity was higher in Narok than in Nakuru County. The *Fusarium* spp. isolated from wheat ears sampled from Narok County in order of decreasing incidence were: *F. avenaceum*, *F. poae*, *F. graminearum*, *F. equiseti*, *F. chlamydosporum*, *F. sambucinum*, *F. tricintcum* and *F. scirpi* (Figure 1A). On the other hand, the *Fusarium* spp. isolated from wheat ears in Nakuru County in decreasing incidence were: *F. graminearum*, *F. avenaceum*, *F. poae*, *F. oxysporum*, *F. crookwellense*, *F. scirpi*, *F. lateritium* and *F. tricinctum* (Table 7). Although *F. avenaceum* and *F. poae* were frequently isolated from wheat ears in both Counties, the incidences were significantly ($p \leq 0.05$) higher in Nakuru than in Narok County.

Fusarium spp. isolated from wheat kernels at harvest from Narok County in decreasing incidence were: *F. tricinctum*, *F. poae*, *F. equiseti*, *F. nivale*, *F. sambucinum*, and *F. graminearum* (Figure 1B). The *Fusarium* species isolated from wheat kernels at harvest from Nakuru County in decreasing incidence were: *F. avenaceum*, *F. graminearum*, *F. poae*, *F. tricinctum*, *F. solani* and *F. culmorum* (Table 8). There was variation in the incidence of various *Fusarium* spp. among the three AEZ in Narok County. *Fusarium avenaceum*, *F. poae* and *F. graminearum* were the most commonly isolated *Fusarium* species from both wheat ears and kernels in the two Counties. However, the diversity of *Fusarium* spp. was higher in the ears than in kernels in both Counties.

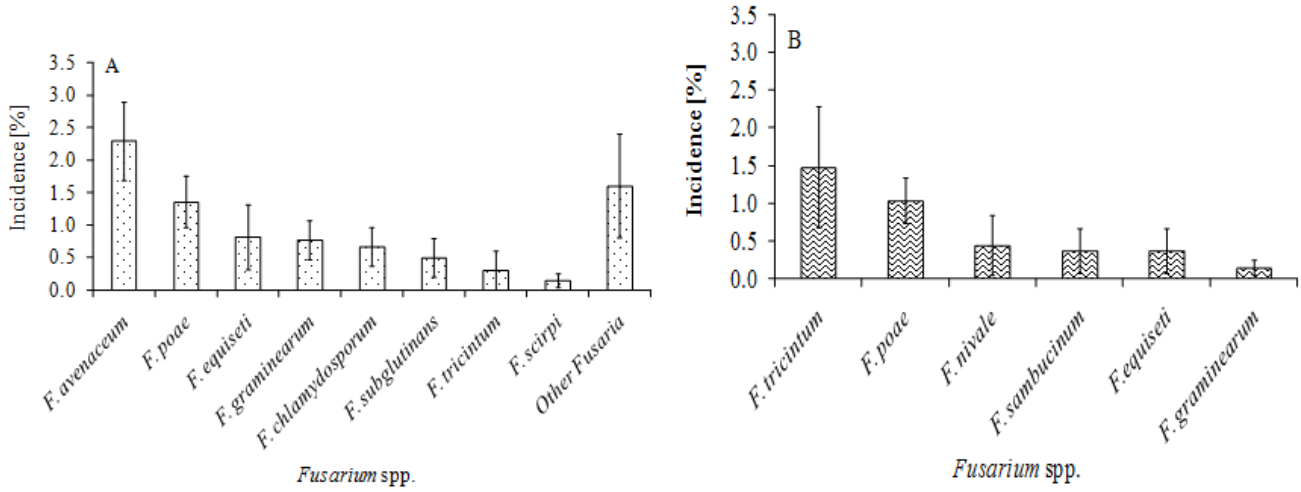

Figure 1. Incidence (%) of *Fusarium* spp. in Spikelets (A) and Wheat Kernels at Harvest (B) in Three AEZs in Narok County, Kenya. Errors bars represent standard errors of means

Table 7. Incidence (%) of *Fusarium* spp. Contaminating Wheat Ears in Different Agro-Ecological Zones in Nakuru County

AEZ	F. gra	F. aven	F. po	F. oxy	F. cro	F. sc	F. lat	F. tric	Others
UH1	0.5b	4.1a	3.1a	3.1a	2.1a	1.3a	0.5ab	0.5a	0.0c
UH2	0.0b	3.6a	1.8a	1.8ab	0.0b	0.6a	0.0b	0.0a	1.1a
UH3	6.7a	3.1a	3.9a	1.9ab	1.2ab	0.8a	1.6a	0.8a	0.3b
LH3	7.9a	0.0b	1.3a	0.0b	0.0b	0.0a	0.0b	0.0a	0.4b
Mean	3.8	2.7	2.5	1.7	0.8	0.6	0.5	0.3	0.4
LSD ($p \leq 0.05$)	3.4	2.9	2.9	2.3	1.6	1.4	1.4	1.1	0.2
CV (%)	27.3	22.0	26.7	26.4	56.0	135	43.4	0.0	43.4

F.gra - F. graminearum, F. aven - F. avenaceum, F. poa - F. poae, F. oxysporum, F. cro – F. crockwelense, F. sc – F. scripi, F. lat – F. lateratium, F. tric - F. tricinctum
Means followed by the same letter within each column are not significantly ($p \leq 0.05$) different.

Table 8. Incidence (%) of *Fusarium* spp. Contaminating Wheat Kernels in Different Agro-Ecological Zones in Nakuru County

AEZ	F. aven	F. gra	F. poa	F. tric	F. so	F. cul	Others[a]
UH1	3.2b	0.6b	0.3b	0.0a	0.0a	0.0a	0.0a
UH2	5.9b	1.1b	0.4b	1.1a	0.0a	0.0a	0.0a
UH3	15.2a	2.5b	0.0b	0.3a	0.7a	0.3a	0.3a
LH3	1.9b	5.9a	4.5a	0.4a	0.0a	0.0a	0.0a
Mean	6.5	2.6	1.3	0.5	0.2	0.1	0.1
LSD ($p \leq 0.05$)	5.6	2.4	1.6	1.1	0.8	0.5	0.5
CV (%)	4.2	36.4	58.7	71.4	155	265.1	265.3

F. aven - F. avenaceum, F.gra - F. graminearum, F. poa - F. poae, F. tric - F. tricinctum, F. so - F. solani, F. cul - F. culmorum
[a] F. oxysporum, F. sambucinum, F. verticillioides.
Means followed by the same letter within each column are not significantly ($p \leq 0.05$) different.

3.5. Contamination of Wheat Kernels with Deoxynivalenol and T2-toxin

All the wheat kernel samples from Narok County were contaminated with T-2 toxin while 5.9% were contaminated with DON (Table 8). T-2 toxin levels ranged from 8.8 to 37.0 µgkg^{-1} (Mean = 25.1 µgkg^{-1}) while levels of DON ranged from below limit of detection to 144.2 µgkg^{-1}. There was variation in DON and T-2 toxin concentrations among wheat samples from different AEZ with the levels of the two toxins being highest in LH3 and lowest in UH3.

The levels of deoxynivalenol in wheat kernels in Nakuru County varied from below the limit of detection to 623 µgkg^{-1} with the highest levels being detected in samples from LH3 and the lowest in samples from UH2 (Table 9). Levels of T-2 toxin varied from <LOD to 69.3 µgkg^{-1}.

Table 9. Concentration (µgkg^{-1}) of Deoxynivalenol and T-2 Toxin in Wheat Kernels Sampled at Harvest from Different Agro-Ecological Zones in Narok and Nakuru Counties

AEZ	Sample size	Deoxynivalenol	T-2 toxin	
		Range	Range	Mean[a]
Narok County (n = 51)				
LH3	15	< LOD – 144.2	13.6 - 37.0	27.4
LH2	18	<LOD	18.3 – 34.3	25.8
UH3	18	< LOD	8.8 – 35.0	22.7
Nakuru County (n = 51)				
UH1	13	<LOD - 321.0	17.7 - 69.3	36.6
UH2	11	<LOD	20.8 - 27.0	24.9
UH3	17	<LOD - 252.7	<LOD - 42.9	-
LH3	10	<LOD - 623.0	19.3 - 30.9	26.5

LOD – limit of detection; LOD for DON = 18.5 µgkg^{-1}; LOD for T2-toxin = 3.5 µgkg^{-1}; [a] Average for T2-toxin levels.

4. Discussion

The prevalence of FHB in Narok and Nakuru Counties was 100%. This concurs with the findings by [17] and [10], who reported incidence of up to 97% and 88% during the 2006 cropping season in Nakuru district, and the 2008 cropping season in Narok, Imenti North and Nyandarua districts in Kenya, respectively. However, in the current study, the mean incidence and severity were relatively low with the disease incidence being higher in Nakuru than in Narok County. This could be related to differences in temperature and relative humidity between the two Counties during the 2013 cropping season. Incidence of FHB in Narok County significantly varied among the AEZs which could be attributed to differences in environmental conditions in the three agro-ecological zones [17,38,39]. The high incidence of FHB in UH3 agro-ecological zone of Narok County could be attributed to high rainfall (1000 - 1200mm) and relative humidity in the AEZ during the 2013 cropping season which might have favored infection of wheat ears by *Fusarium* spp. [17,38,39].

Overall, the relatively low incidence and severity of FHB in the current study could be attributed to non-conducive weather conditions in the two Counties. Temperature and relative humidity in Narok and Nakuru Counties were relatively low during the 2013 cropping season. The temperature and relative humidity between June and August 2013, the period when wheat in Narok County was at flowering stage, ranged from 9.3 - 22.7 °C and 49.7 – 79.3%, respectively (Appendix A). In Nakuru County, temperature and relative humidity between August and October 2013, ranged from 11.1 - 28.5 °C and 24.0 - 84.0%, respectively (Appendix B). Infection of wheat by FHB is favored by a temperature range of 15 - 30 °C and relative humidity greater than 90% [14]. Infection is also accelerated by precipitation immediately before and after anthesis [40,41].

The major fungal pathogens isolated from wheat ears and kernels in Narok County were *Epicoccum* spp., *Alternaria* spp. and *Chaetomium* spp. while *Fusarium* spp., *Epicoccum* spp. and *Alternaria*, spp. were the major fungal pathogens isolated from similar samples in Nakuru County. Co-occurrence of different fungal pathogens could have synergistic effect on severity of FHB and result in excess yield reduction [17,42]. The high

incidence of *Epicoccum* spp. and *Alternaria* spp. in this study concurs with the findings by various researchers [5,17,43,44]. As noted in previous studies, there was a negative correlation between the incidence of *Epicoccum* spp. and *Fusarium* spp. in wheat ears and kernels. A study by [45] showed that *Epicoccum nigrum* has antagonistic properties against *F. avenaceum*, *F. graminearum* and *F. oxysporum*. This could therefore explain the inverse relationship in the incidence of *Fusarium* spp. and *Epicoccum* spp. in the current study. A negative correlation was also observed between the incidence of *Fusarium* spp and *Alternaria* spp. This could explain the high incidence of *Fusarium* spp. in Nakuru County where the incidence of *Alternaria* spp. was low while in Narok County, the incidence of *Fusarium* spp. was low with a corresponding high incidence of *Alternaria* spp. *Epicoccum* spp. and *Alternaria* spp. are saprophytes that cause grey or black discoloration of the wheat heads resulting in sooty moulds, black point or smudge [45,46]. Some *Alternaria* spp. are known to produce alternariol, alteneune, tenuazonic acid and altertoxins [44,47] while *Alternaria alternata* causes huge losses in yields [48].

Over fourteen *Fusarium* spp. were isolated from wheat ears and kernels in the two Counties. Previous studies in Kenya reported similar diversity but varying predominance of *Fusarium* spp. [5,10,17]. Similar to findings by [5], the mean incidence of *Fusarium* spp. was significantly higher in wheat ears than in harvested kernels in both Counties. This suggests superficial infection of wheat by FHB pathogens. The wide diversity of *Fusarium* spp. confirmed that in Kenya FHB of wheat is caused by a complex of *Fusarium* spp. [5,10]. In Narok County, the main *Fusarium* spp. isolated from wheat ears were *F. avenaceum*, *F. poae*, and *F. graminearum* while *F. tricinctum* and *F. poae* were the predominant species in kernels. In Nakuru County, *F. graminearum*, *F. avenaceum* and *F. poae*, which are the main causative agents of FHB, were the major *Fusarium* spp. isolated from both wheat ears and kernels. Co-occurrence of these major FHB causal pathogens implies a risk of co-occurrence of mycotoxins in wheat kernels if the fungi are not controlled.

The relatively high incidence of *F. poae* and *F. tricinctum*; and *F. graminearum* and *F. avenaceum* in harvested wheat kernels in Narok and Nakuru Counties, respectively implies that they are important components of the *Fusarium* spp. complex causing FHB and consequently may have played a major role in contamination of harvested wheat with mycotoxins mainly T2-toxin and DON. This concurs with previous studies that associated the type of *Fusarium* spp. isolated from wheat kernels to the type of mycotoxin detected [5,17]. *Fusarium poae* is a cosmopolitan species but more common in temperate regions while *F. tricinctum* is a temperate species hence its high occurrence in UH3 in Narok County.

T-2 toxin and DON were detected in 100% and 5.9% of the wheat kernels in Narok County, respectively although the concentration of the two toxins was relatively low. In Nakuru County, 47% and over 90% of samples were contaminated with DON and T-2 toxin, respectively. Detection of these toxins in wheat concurs with previous reports in Kenya [5,15]. However, the levels contrasted the findings by [5] who reported high concentration of DON of up to 1310 μgkg^{-1} in wheat sampled from Nakuru County. Deoxynivalenol is a potent mycotoxin mainly produced by *F. graminearum* and *F. culmorum* and is a virulent factor for *F. graminearum* [22,49]. Although DON is less toxic compared to other trichothecenes, it is the most commonly detected *Fusarium* toxin and most economically important mycotoxin of small grain cereals [6]. T-2 toxin poses serious threats to human and animal health including weight loss, poor weight gain, bloody diarrhea, and decreased animal productivity [26,50].

Levels of T-2 toxin and DON in wheat kernels in this study were within the limits set by the European Commission (EC) and the United States Food and Drug Administration (FDA). Kenya does not have standards for DON and T-2 toxin. The low levels of T-2 toxin and DON could be attributed to the relatively low incidence of *Fusarium* spp. in wheat ears and kernels. Infection of wheat ears and kernels by *Fusarium* spp. could also have been superficial. There is also a possibility that the isolated *Fusarium* spp. produced toxins other than DON and T-2 toxin targeted in the current study. Environmental factors influencing the development of FHB also influence accumulation of the toxins [6]. The low temperature, rainfall and relative humidity in Narok and Nakuru Counties during the 2013 cropping season could therefore be contributing factors to the relatively low levels of the two toxins.

5. Conclusions

This study provided baseline information on the incidence and severity of FHB in Narok and Nakuru Counties. Based on these findings, there was low incidence of *Fusarium* spp. associated with FHB as well as low levels of DON and T-2 toxin in wheat harvested during the 2013 cropping season in the two Counties. However, monitoring and surveillance programs for FHB, DON and T-2 toxin levels are necessary as their occurrence varies among seasons. The high incidence of other fungal species that contribute to yield losses underscore the need for disease management programs that consider the role of multiple fungi in wheat grain quality, safety and yield losses.

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Competing Interests

The Authors have no competing interest.

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Supplementary Data

Appendix A. Monthly Precipitation (mm), Temperature (°C), and Relative Humidity (%) Recorded at the Narok Meteorological Weather Station in 2013

Month	Total Precipitation	Minimum temperature	Maximum temperature	Relative humidity 06Z	Relative humidity 12Z
January	63.4	10.5	25.7	74.0	46.0
February	73.6	9.3	26.6	70.0	37.0
March	103.4	11.7	26.6	74.0	41.0
April	240.7	13.7	23.8	87.0	63.0
May	57.0	12.2	22.4	86.0	60.0
June	2.7	9.7	22.0	76.0	53.0
July	16.4	8.7	23.3	81.0	47.0
August	14.9	9.4	22.7	81.0	49.0
September	75.1	10.5	25.4	73.0	43.0
October	Nil	10.2	25.9	65.0	34.0
November	12.7	12.9	26.0	72.0	39.0
December	80.5	11.7	25.0	78.0	52.0

Relative humidity 06Z – relative humidity taken at 9.00am; relative humidity 12Z - relative humidity taken at 3.00pm
Source: Ministry of Environment, Water and Natural Resources, State Department of Environment, Meteorological Service, Kenya (2014).

Appendix B. Monthly Temperature (°C), Precipitation (mm), and Relative Humidity (%) Data Recorded at the Nakuru Meteorological Weather Station in 2013

Month	Total Precipitation	Minimum temperature	Maximum temperature	Relative humidity 06Z	Relative humidity 12Z
January	27.0	11.8	26.7	35.0	72.0
February	0.8	11.1	28.5	24.0	65.0
March	66.5	12.5	28.4	38.0	70.0
April	253.1	13.3	25.1	57.0	84.0
May	80.5	12.6	25.0	49.0	79.0
June	165.7	12.6	24.1	54.0	83.0
July	174.3	12.0	24.1	50.0	82.0
August	113.4	13.9	23.9	50.0	81.0
September	144.1	12.3	25.8	47.0	78.0
October	60.5	11.6	25.9	46.0	70.0
November	62.7	12.3	24.7	60.0	78.0
December	84.9	12.3	24.7	48.0	77.0

Relative humidity 06Z – relative humidity taken at 9.00am; relative humidity 12Z - relative humidity taken at 3.00pm.
Source: Ministry of Environment, Water and Natural Resources, State Department of Environment, Meteorological Service, Kenya (2014).