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Grain mould fungi of sorghum caryopses in Benishangul Gumuz, Ethiopia

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Abstract

Sorghum is one of the staple food crops in Ethiopia. However, several grainmould fungi challenge sorghum production. Graina mould fungi invasion of sorghum caryopses was studied on potato dextrose agar (PDA) and Blotter techniques of ISTA for seventy-two sorghum grain samples collected in Assosa and Metekel Zones of Benishangul Gumuz regional state, Western Ethiopia. Thirty-two fungal species were identified and grouped into nineteen genera from sorghum grain sampled in Assosa and Metekel zones during 2018 and 2019. The most frequently recovered and predominant fungal genera were *Fusarium*, *Epicoccum*, *Exserohilum*, *Cladosporium*, and *Aspergillus*. The results revealed that *F. moniliforme, E. nigrum, E. turcicum, C. lunata*, *C. sphaerospermum, F. solani, A. alternata*, and *Cylindrocarpon* spp. were the most frequent and predominate fungal species associated with sorghum grain mould in the region. The PDA was found to be the best medium for isolating mycoflora of sorghum caryopses.

Keywords – Blotter – Frequency of isolation – Mould fungi – PDA – Relative density – *Sorghum bicolour*

Introduction

Sorghum, *Sorghum bicolor* (L.) Moench belonging to the Poaceae family is the fifth most-produced cereal crop next to maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) in the globe (FAO 2022). It is the third produced cereal crop after maize and rice in Africa. Ethiopia is the second-largest sorghum producer after Nigeria in Africa (FAO 2022). In Ethiopia, sorghum is the third in area allotted behind maize and teff (*Eragrostis tef* (Zucc.) Trotter), and the fourth in tones of production next to maize, teff, and wheat (CSA 2021). All regions in Ethiopia cultivated sorghum crops, but most popularly, Oromia, Amhara, Tigray, SNNP, Somali, and Benishangul Gumuz regions have grown it. In the Benishangul Gumuz region, the Assosa Zone attributed 67.80% hectares, while 11.45% hectares were by Metekel Zone (CSA 2021). The crop is also one of the food security grains of households in the region. It is mainly grown for grains (human consumption) and forage (animal feed), and also for bio-fuels (Britannica 2019).

However, many factors such as drought, poor soil fertility, birds, insect pests, *Striga*, diseases, lack of modern agricultural equipment, and insignificant use of external inputs (improved varieties, fertilizers, and certified seeds) affect sorghum production in Eastern Africa, including

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Ethiopia (Wortmann et al. 2006, Amelework et al. 2016, Dorcas et al. 2019, Mwamahonje et al. 2021). Besides, diseases such as *Colletotrichum sublineolum* and grain mould diseases pose a serious constraint to sorghum production in Ethiopia (Mengistu et al. 2018, Mengistu et al. 2019), particularly in Benishangul Gumuz region where there is favourable weather condition – subtropical wet, humid climate characterized by tropical wet and high temperatures (Gonfa 1996) and prevailing prolonged rainy season spreads through May to October (http://www.ethiopar.net) – for disease development. This favors the development of grain mould fungi in sorghum and other cereal crops grown in the area.

Sorghum grain mould caused complex fungal pathogens (Navi et al. 1999, Tarekegn et al. 2006). The disease is one of the crucial challenges for high-quality sorghum grain production (Thakur et al. 2006, Sharma et al. 2010). The mould pathogens adversely affect sorghum grains via grain discoloration, endosperm deterioration (breakdown), and production of mycotoxins, which are harmful to human & animal health (Leslie 2014, Lahouar et al. 2015). Mould fungi infection is favoured by prolonged periods of humid and/or rainy weather and temperatures of 25 °C to 35 °C during grain development (Garud et al. 2000, Navi et al. 2005, Thakur et al. 2006). Grain mould can cause losses from 30% to 100% based on cultivar, period of flowering and weather conditions during the following harvest (Singh & Bandyopadhyay 2000).

So far, there are no past studies conducted in the region to document grain mould fungi associated with sorghum grains. This information can help in looking for new ways for sorghum improvement. Thus, this study aimed to identify and document grain mould fungi of sorghum in Benishangul Gumuz Region, Western Ethiopia.

Materials & Methods

Description of the study area

The study area is the Assosa zone and Metekel zone of the Benishangul Gumuz Regional state of Ethiopia, where high humidity (77.06% to 85.5%), and high temperature (29.1 to 32.6 °C) prevail during sorghum flowering and grain setting (https://tcktcktck.org/ethiopia/benshangul-gumaz/asosa) (Fig. 1). These further exacerbated the occurrence of grain mould and foliar diseases in sorghum. We collected samples from Assosa, Bambasi and Kurmuk districts in the Assosa zone, and Bulen, Dangur, Guba, Mandura and Pawi districts in the Metekel Zone (Fig. 1).

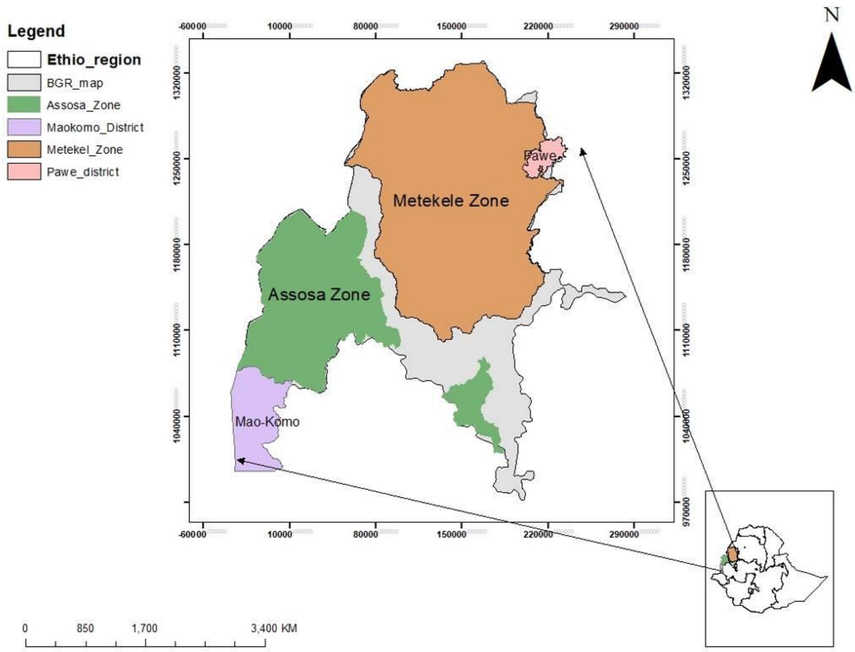

Fig. 1 – Map of the study area.

Sample collection and fungal isolation

Seventy-two sorghum grain samples (each with grain weight of 300 g) were collected from local stores and farm fields in Assosa and Metekel zones during 2018-2019 (Table 1). We conducted the fungal invasion study following agar and moist blotter plate methods. Forty sorghum grains per sample were surface disinfected in 3% sodium hypochlorite for 2 minutes and subsequently rinsed trice in sterile distilled water. Then, the grains were drained under a laminar flow hood and 10 grains were aseptically placed on solidified potato dextrose agar (PDA) media amended with 250 gm chloramphenicol per liter for inhibition of bacterial contaminants. The other 10 grains were placed onto each plate containing sterile distilled water-soaked filter papers. Both methods were replicated twice and incubated at 25 °C for 5 to 10 days. The seed-borne fungi found on each and every grain were isolated and purified.

Table 1 Composite grain samples used to study sorghum grain mould fungi.

Growing region	Growing zone	Year	Amount (g)
Benishangul Gumuz	Assosa	2018	300
	Metekel	2018	300
	Assosa	2019	300
	Metekel	2019	300

Morphological analysis

Fungal colonies grown on sorghum grains were observed under stereo-binocular microscope and representative isolates of fungal species were transferred onto Potato Dextrose Agar (PDA) medium to study the macro- and micro- morphological characteristics. All the isolates of fungal species were identified up to the species levels using keys and manuals (Navi et al. 1999, Mathur & Kongsdal 2003, Leslie & Summerell 2008).

Data collected

Percent of sorghum grains colonized by fungi, germinated sorghum grain, frequency, relative abundance, density of fungal species identified data were recorded. The isolation frequency and relative density of genera and species were calculated according to Ghiasian et al. (2004) as follows:

$$Frequency\ (\%) = \frac{Number\ of\ samples\ in\ which\ a\ genus\ or\ species\ occurred}{Total\ number\ of\ samples}\ x\ 100$$

$$Relative\ density\ (\%) = \frac{Number\ of\ isolates\ of\ a\ genus/species}{Total\ number\ of\ fungi/\ genus\ isolates}\ x\ 100$$

Statistical analysis

Percentage germination, health seed, and infected seed were analyzed using SAS 9.3 statistically software (SAS 2010). The data obtained were analyzed statistically and significance was calculated at $p < 0.05$ levels of probability. Whereas frequency of fungal isolation, and relative density of fungal species.

Results

Physical inspection of sorghum grains

The analysis of variance revealed that percentages of healthy sorghum grains and germinated sorghum grains were statistically showed significant difference (at $P < 0.01$) among the years of sample collection, and districts from where the samples were collected. At the same time, percentages of sorghum grains infected with mould fungi had shown a varying difference (at $P < 0.05$) between years, districts, and sorghum varieties (Table 2).

Table 2 ANOVA table for mean squares of healthy grain, infected grain, and germinated grain.

Source	Degree of freedom	Healthy grains (%)	Infected grains (%)	Germinated grains (%)
Years	1	6961.29**	2135.34**	2953.61**
Zones	1	2135.34^{NS}	2135.34^{NS}	9.50^{NS}
Districts	6	645.15**	528.43*	680.13**
Varieties	2	895.56^{NS}	895.56*	19.04^{NS}
Mean		**31.87**	**68.13**	**93.70**
CV (%)		**65.20**	**30.50**	**8.75**

^{NS} not significant at p<0.05; ** significant at p < 0.01; * significant at p < 0.05

The results indicated that most of the sorghum grains sampled in 2018 were healthier than those sampled in 2019 cropping season of Ethiopia. On the other hand, percentages of infected sorghum grains were higher in 2019 than in 2018 both on PDA and blotter techniques. However, the percentages of germinated sorghum grains were higher in 2019 than in 2018 (Table 3). Results showed no statistical differences in percentages of healthy sorghum grains and mould fungi infected sorghum grains at Zone level, but Assosa zone recorded a higher percentage of germinated sorghum grains on blotter method and lower in PDA (Table 3). At district level, most of the healthier sorghum grains were obtained from Bulen and Mandura (in Metekel zone), and Kurmuk and Assosa (in Assosa zone). The most mould fungi infected sorghum grains were founded from Guba, Dangur, and Pawi in Metekel zone and Bambasi and Assosa in Assosa zone (Table 3).

This study found that sorghum variety Adukara was highly infected by mould fungi followed by Assosa–1 and the local cultivars. The higher germination percentage was also recorded by local cultivars on both blotter and PDA methods. Assosa–1 showed a higher germination percentage on blotter method than Adukara, but it become lower in percentage of germinated grains on PDA method (Table 3).

Morphological identification of grain mould fungi of sorghum

From both PDA and blotter methods, 32 fungal species had identified and grouped into 19 genera from sorghum grains sampled in 2018 and 2019 across Assosa and Metekel Zones of Benishangul Gumuz, Ethiopia (Table 5). *Fusarium* and *Epicoccum* were the most frequently isolated and relatively dominant genera on PDA in 2018. While, *Fusarium*, *Exserohilum*, and *Trichoderma* were the most frequent and comparatively dominant genera, followed by *Cladosporium*, *Aspergillus*, and *Epicoccum* on the blotter technique (Table 4). Likewise, *Fusarium* and *Epicoccum* were the most frequently detected and predominant genera on PDA in 2019. Also, *Fusarium*, *Cladosporium*, *Epicoccum*, and *Exserohilum* were the most frequent and comparably dominant genera on the blotter technique in 2019 (Table 4).

Twenty-one fungal species in 2018 and 32 fungal species in 2019 had recovered from sorghum grains collected across Assosa and Metekel Zones of Benishangul Gumuz, Ethiopia (Table 5). In 2018, *F. moniliforme*, *E. nigrum*, and *F. solani* were the most frequently recovered and relatively dominant species on PDA, while *F. moniliforme, E. turcicum*, and *Trichoderma* spp. were the most commonly recovered and predominant species on blotter technique (Table 5).

In 2019, the highest frequency (93.55) and relative dominance (17.59) had recorded for *F. moniliforme* followed by *E. nigrum* (56.45 and 40.21), *E. turcicum* (51.61 and 5.82), *C. lunata* (45.16 and 5.42), *A. alternata* (45.16 and 4.37) on PDA, whereas the most frequently and dominantly recorded fungal species on blotter technique were *E. turcicum, C. sphaerospermum, F. moniliforme, and Cylindrocarpon* spp. (Table 5).

On PDA, *F. moniliforme* and *E. nigrum* were the most frequently and predominately identified species followed by *E. turcicum* in Assosa Zone, while the highest frequency (90.24) and relative dominance (21.20) had recorded for *F. moniliforme* followed by *C. lunata*, and *E. nigrum* in Metekel Zone (Table 6).

On blotter technique, the most frequent and relatively dominant fungi were *F. moniliforme* followed by *C. sphaerospermum* (93.33 and 15.38), *E. turcicum* (90 and 15.96), *G. sorgina* (70 and 12.88), and *E. nigrum* (60 and 10) in Assosa Zone, while *E. turcicum* (100 and 20.41), *F. moniliforme* (100 and 20.07), *C. sphaerospermum* (92.68 and 18.03), and *G. sorghi* (73.17 and 9.69) were the most frequent and relatively dominant in Metekel Zone (Table 6).

In Assosa Zone, 25 fungal species grouped under 18 genera had associated with sorghum grain mould (Table 6 and Table 7). Most of the Assosa Zone fungal isolates had recovered from sorghum grains sampled from Assosa and Bambasi districts (Table 7). On PDA, the most frequent and relatively dominant ones were *E. nigrum*, *F. moniliforme*, *F. solani* in Assosa district *F. moniliforme*, *C. sphaerospermum*, and *E. turcicum* in the Bambasi district, and *F. moniliforme* and *E. turcicum* in Kurmuk district. On the blotter technique, the most commonly and dominantly recovered species were *G. sorghi*, *E. nigrum*, *P. sorghina*, *C. sphaerospermum*, *F. moniliforme*, *E. turcicum*, and *S. obovata* in Assosa district, *C. sphaerospermum*, *E. turcicum*, *F. moniliforme*, *E. nigrum*, *G. sorghi*, and *A. alternata* in Bambasi district, and *F. moniliforme*, *E. turcicum*, and *C. sphaerospermum* in Kurmuk district (Table 7).

In Metekel Zone, 31 fungal species categorized under 18 genera had recovered from sorghum grain (Table 6, 8), and most of the recovered isolates were from Pawi, Guba, and Dangur districts. The most commonly recovered and predominant fungal species from samples of Bulen were *F. moniliforme*, *E. nigrum*, *A. alternata*, and *P. wax* on PDA, while *F. moniliforme*, *E. turcicum*, *Trichoderma* spp., *E. nigrum*, and *C. sphaerospermum* on blotter (Table 6).

In Mandura district, *F. moniliforme*, *E. nigrum*, and *F. solani* were the most frequent and relatively dominant fungal species on PDA, whereas *F. moniliforme*, *Trichoderma* spp., *A. flavus*, *E. nigrum*, and *E. turcicum* on blotter method. The Pawi district's most frequent and relatively dominant species were *F. moniliforme*, *A. alternata*, *C. sphaerospermum*, and *F. solani* on PDA, while *F. moniliforme*, *C. sphaerospermum*, *E. turcicum*, and *G. sorghi* on blotter method.

Table 3 Effect of plating method on seed health and germination of grains.

Class	**Variables**	**Proportions (%)**	**Healthy grain (%)**		**Infected grain (%)**		**Germinated grain (%)**	
			Blotter	**PDA**	**Blotter**	**PDA**	**Blotter**	**PDA**
Year	2018	16.44	53.89^{a}	6.67	46.11^{b}	93.33	75.00^{b}	70.00
	2019	83.56	27.54^{b}	2.42	72.46^{a}	97.58	97.38^{a}	79.36
LSD			**13.12**	**4.68**	**13.12**	**4.67**	**5.18**	**13.89**
Variety	Assosa–1	4.11	31.11^{ab}	0.00	68.89^{ab}	100.00	97.78	46.67^{b}
	Adukara	2.74	0.00^{b}	0.00	100.00^{a}	100.00	93.34	50.00^{b}
	Local	93.15	32.84^{a}	3.18	67.16^{b}	96.82	93.53	80.45^{a}
LSD			**31.23**	**9.87**	**31.23**	**9.87**	**12.33**	**28.36**
Zones	Assosa	41.10	33.56	2.67	66.45	97.33	97.78^{a}	72.67^{b}
	Metekel	58.90	30.70	3.17	69.30	96.83	90.85^{b}	82.19^{a}
LSD			**9.88**	**3.15**	**9.88**	**3.15**	**3.90**	**9.06**
Districts	Assosa	13.70	31.33^{abc}	5.00^{ab}	68.67^{abc}	95.00^{ab}	98.00^{ab}	72.00^{ab}
	Bambasi	13.70	24.67^{cd}	0.00^{b}	75.33^{ab}	100^{a}	96.00^{ab}	65.00^{b}
	Kurmuk	13.70	44.67^{abc}	3.00^{ab}	55.34^{bcd}	97.00^{ab}	99.33^{a}	81.00^{ab}
	Bulen	9.59	59.05^{a}	8.00^{a}	40.95^{d}	92.00^{b}	89.52^{b}	82.00^{ab}
	Dangur	13.70	26.00^{cd}	0.00^{b}	74.00^{ab}	100^{a}	98.00^{ab}	84.00^{ab}
	Guba	15.07	13.33^{d}	0.00^{b}	86.67^{a}	100^{a}	95.15^{ab}	88.18^{a}
	Mandura	2.74	53.34^{ab}	10.00^{a}	46.67^{cd}	90.00^{b}	43.34^{c}	65.00^{b}
	Pawi	17.81	30.26^{bcd}	5.39^{ab}	69.74^{ab}	94.62^{ab}	89.74^{b}	78.46^{ab}
LSD			**22.85**	**7.88**	**22.85**	**7.28**	**9.02**	**21.93**

Key: LSD = least significant difference; PDA = Potato dextrose agar

In Dangur, the most frequent and comparatively dominant fungal species were *F. moniliforme* and *C. lunata* on PDA, whereas *C. sphaerospermum*, *E. turcicum*, *G. sorghi*, and *F. moniliforme* on blotter method. In the Guba district, *F. moniliforme*, *A. alternata*, *C. lunata*, *E. nigrum*, and *E. turcicum* were the most commonly and dominantly recovered fungal species on PDA, while *F. moniliforme*, *E. turcicum*, *C. sphaerospermum*, *C. lunata*, *G. sorghi*, and *A. alternata* were the most frequently and dominantly detected fungi on the blotter technique (Table 8).

Table 4 Fungal genera associated with sorghum grain mould in Assosa and Metekel Zones of Benishangul Gumuz, Ethiopia in 2018 and 2019.

Genera	**2018**						**2019**					
	PDA			**Blotter**			**PDA**			**Blotter**		
	N	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**
Acremonium	2	11.11	3.45				6	6.35				
Alternaria	3	33.33	5.17				33	44.44	4.37	47	40.32	4.84
Aspergillus	3	22.22	5.17	10	50	7.30	19	20.63	2.51	27	17.74	2.78
Bipolaris										1	1.61	0.10
Botrytis										5	6.45	0.51
Cladosporium				10	70	7.30				176	95.16	18.13
Curvularia	3	11.11	5.17	2	20	1.46	54	50.79	7.14	44	32.26	4.53
Chaetomium										2	3.23	0.21
Colletotrichum										6	3.23	0.62
Cylindrocarpon	2	22.22	3.45				11	15.87	1.46			
Epicoccum	12	66.67	20.67	9	60	6.57	241	55.56	40.21	65	45.16	6.69
Exserohilum	1	11.11	1.11	22	100	16.06	49	53.97	6.48	192	93.55	19.77
Fusarium	26	88.89	44.83	45	100	32.85	192	93.65	25.40	152	96.77	15.65
Gloecercospora				5	40	3.65				119	75.81	12.26
Penicillium	4	22.22	6.90	4	30	2.92	22	25.40	2.91	18	22.58	1.85
Phoma				3	20	2.19				58	41.94	5.97
Spadicoides				1	10	0.73				33	25.81	3.40
Trichoderma	1	11.11	1.72	22	80	16.06	4	6.35	0.53	18	25.81	1.85
Rhizopus	1	11.11	1.72	4	20	2.92	8	4.76	1.06	8	8.06	0.82
Total isolates	**58**			**137**			**693**			**971**		
Sub–total	**195**						**1664**					
Overall isolates	**1859**											

Key: N = detection number; PDA = Potato dextrose agar

Table 5 Mycoflora of sorghum caryopses in Assosa and Metekel Zones of Benishangul Gumuz, Ethiopia in 2018 and 2019.

Genera	2018						2019					
	PDA			Blotter			PDA			Blotter		
	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)
Acremonium strictum W.Gams	2	11.11	3.45				6	6.45	0.79			
Alternaria alternata (Fr.) Keissl.	3	33.33	5.17				33	45.16	4.37	38	39.34	3.91
Alternaria brassicicola (Schwein.) Wiltshire										4	1.64	0.41
Alternaria longipes (Ellis & Everh.) E.W. Mason										3	3.28	0.31
Alternaria tenuissima Samuel Paul Wiltshire										2	3.28	0.21
Aspergillus flavus Link	3	22.22	5.17	10	50.00	7.30	5	8.06	0.66	25	16.39	2.57
Aspergillus niger (Tiegh.) Speg.							14	14.52	1.85	2	1.64	0.21
Bipolaris sacchari (E.J. Butler) Shoemaker										1	1.64	0.10
Botrytis cinerea Pers.										5	6.56	0.51
Chaetomium oryzae										2	3.28	0.21
Cladosporium sphaerospermum Penz.				10	70.00	7.30	36	35.48	4.76	176	96.72	18.13
Colletotrichum graminicola (Ces.) G.W Wilson							18	22.58	2.38	6	3.28	0.62
Curvularia australiensis (DRECAU)							4	6.45	0.53			

Table 5 Continued.

Genera	2018						2019					
	PDA			Blotter			PDA			Blotter		
	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)	N	Frequency (%)	Relative density (%)
Curvularia lunata (Wakker) Boedijn	2	11.11	3.45	2	20.00	1.46	41	45.16	5.42	40	29.51	4.12
Curvularia pallescens Boedijn	1	11.11	1.72				9	12.90	1.19	4	3.28	0.41
Cylindrocarpon spp.	2	22.22	3.45				11	16.13	1.46			
Epicoccum nigrum Link	12	66.67	20.69	9	60.00	6.57	241	56.45	40.21	65	45.90	6.69
Exserohilum rostratum K.J. Leonard							5	8.06	0.66	11	11.48	1.13
Exserohilum turcicum (Pass.) K.J. Leonard & Suggs	1	11.11	1.72	22	100.00	16.06	44	51.61	5.82	181	95.08	18.64
Fusarium moniliforme Sheldon	16	77.78	27.59	45	100.00	32.85	133	93.55	17.59	148	98.36	15.24
Fusarium oxysporium Schlecht. Emend. Snyder & Hansen	1	11.11	1.72				17	17.74	2.25			
Fusarium semitectum Berk. & Ravenel							6	8.06	0.79	4	6.56	0.41
Fusarium solani (Mart.) Sacc.	8	55.56	13.79				36	41.94	4.76			
Fusarium verticillioides (Sacc.) Nirenberg	1	11.11	1.72									
Gloecercospora sorghi D.C. Bain & Edgerton				5	40.00	3.65				119	77.05	12.26
Penicillium citrinum Thom, C.	1	11.11	1.72				5	8.06	0.66	8	11.48	0.82
Penicillium digitatum (Pers.) Sacc.				4	30.00	2.92	3	3.23	0.40	10	13.11	1.03

Table 5 Continued.

Genera	2018						2019					
	PDA			Blotter			PDA			Blotter		
	N	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**
Penicillium wax	3	11.11	5.17				14	17.74	1.85			
Phoma sorghina				3	20.00	2.19				58	42.62	5.97
Rhizopus stolonifer Vuillemin	1	11.11	1.72	4	20.00	2.92	8	4.84	1.06	8	8.20	0.82
Spadicoides obovata (Cooke & Ellis) S. Hughes				1	10.00	0.73				33	26.23	3.40
Trichoderma spp.	1	11.11	1.72	22	80.00	16.06	4	6.45	0.53	18	26.23	1.85
Total detection	**58**			**137**			**693**			**971**		
Overall isolates	**1859**											

Key: N = number of detections

Table 6 Fungal species associated with sorghum caryopses in two zones of Benishangul Gumuz, Ethiopia during 2018 and 2019.

Genera	2018						2019					
	PDA			Blotter			PDA			Blotter		
	Assosa Zone			Metekel Zone			Assosa Zone			Metekel Zone		
	N	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**
Acremonium strictum	1	3.33	0.27	7	9.76	1.83				1	2.44	0.17
Alternaria alternata	11	26.67	2.98	25	56.10	6.54	14	36.67	2.69	24	31.71	4.08
Alternaria brassicicola										4	2.44	0.68
Alternaria longipes										3	4.88	0.51
Alternaria tenuissima							1	3.33	0.19	1	2.44	0.17
Aspergillus flavus	5	16.67	1.36	3	4.88	0.79	24	30.00	4.62	11	14.63	1.87
Aspergillus niger	6	10.00	1.63	8	14.63	2.09	2	3.33	0.38			
Bipolaris sacchari											2.44	0.17
Botrytis cinerea							5	13.33	0.96			

Table 6 Continued.

Genera	**2018**						**2019**					
	PDA			**Blotter**			**PDA**			**Blotter**		
	Assosa Zone			**Metekel Zone**			**Assosa Zone**			**Metekel Zone**		
	N	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**
Chaetomium oryzae							1	3.33	0.19	1	2.44	0.17
Cladosporium sphaerospermum	*22*	40.00	5.96	14	24.39	3.66	80	93.33	15.38	106	92.68	18.03
Colletotrichum graminicola	*7*	16.67	1.90	11	21.95	2.88				6	4.88	1.02
Curvularia australiensis	*2*	6.67	0.54	2	4.88	0.52						
Curvularia lunata	8	26.67	2.17	35	51.22	9.16	14	30.00	2.69	28	26.83	4.76
Curvularia pallescens	*1*	3.33	0.27	9	19.51	2.36				4	4.88	0.68
Cylindrocarpon spp.	1	3.33	0.23	12	26.83	3.14						
Epicoccum nigrum	176	86.67	47.70	77	36.59	20.16	52	60.00	10.00	22	39.02	3.74
Exserohilum rostratum				5	12.20	1.31				11	17.07	1.87
Exserohilum turcicum	*22*	56.67	5.96	23	39.02	6.02	83	90.00	15.96	120	100.00	20.41
Fusarium moniliforme	68	93.33	18.43	81	90.24	21.20	75	96.67	14.42	118	100.00	20.07
Fusarium oxysporium				18	29.27	4.71						
Fusarium semeticum	4	10.00	1.08	2	4.88	0.52	1	3.33	0.19	3	7.32	0.51
Fusarium solani	18	43.33	4.88	26	43.90	6.81						
Fusarium verticillioides				1	2.44	0.26						
Gloecercospora sorghi							67	70.00	12.88	57	73.17	9.69
Penicillium citrinum	1	3.33	0.27	5	12.20	1.31	8	23.33	1.54			
Penicillium digitatum	*3*	6.67	0.81				9	23.33	1.73	5	9.76	0.85
Penicillium wax	5	13.33	1.36	12	19.51	3.14						
Phoma sorghina							45	53.33	8.65	16	29.27	2.72
Rhizopus stolonifer	7	6.67	1.90	2	4.88	0.52	5	10.00	0.96	7	9.76	1.19
Spadicoides obovata							22	30.00	4.23	12	19.51	2.04
Trichoderma spp.	1	3.33	0.27	4	9.76	1.05	12	33.33	2.31	28	34.15	4.76
Total isolates	**369**			**382**			**520**			**588**		
Overall isolates	**1859**											

Key: N = number of detections; PDA = Potato dextrose agar

Table 7 Fungal species associated with sorghum grain mould in Assosa Zone by districts, Benishangul Gumuz, Ethiopia during 2018 and 2019.

Fungal species	**Assosa**			**Bambasi**			**Kurmuk**		
	N	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**	**N**	**Frequency (%)**	**Relative density (%)**
Acremonium strictum		–	–	1	– (10)	– (0.6)			
Alternaria alternata	10	50 (30)	2.7 (2.9)	14	60 (40)	4 (3.6)	1	– (10)	– (1.3)
A. tenuissima				1	10 (–)	0.5 (–)			
Aspergillus flavus	8	30 (40)	1.8 (2.9)	14	50 (–)	7 (–)	7	10 (10)	6 (1.3)
Aspergillus niger	6	10 (10)	0.9 (2.9)	2	– (20)	– (1.0)			
Botrytis cinerea	3	30 (–)	1.4 (–)	2	10 (–)	1 (–)			
Chaetomium oryzae				1	10 (–)	0.5 (–)			
Cladosporium sphaerospermum	24	100 (40)	6.9 (6.6)	60	100 (80)	23.4 (7.8)	18	80 (–)	18 (–)
Colletotrichum graminicola	1	– (10)	– (0.7)	6	– (40)	(3.6)			
Curvularia australiensis				2	– (20)	– (1.2)			
Curvularia lunata	11	40 (40)	3.2 (2.9)	10	50 (30)	3.5 (1.8)	1	– (10)	– (1.3)
Curvularia pallescens				1	– (10)	– (0.6)			
Cylindrocarpon spp.							1	– (10)	– (1.3)
Epicoccum nigrum	87	90 (100)	15.1 (39.7)	98	80 (100)	8.5 (48.8)	106	10 (100)	2 (67.5)
Exserohilum turcicum	31	90 (50)	11.4 (4.4)	44	100 (70)	16.9 (6)	30	80 (50)	24 (7.5)
Fusarium moniliforme	49	90 (100)	10 (18.4)	50	100 (90)	10 (18.1)	44	100 (90)	31 (16.3)
Fusarium semitectum				5	10 (30)	0.5 (2.4)			
Fusarium solani	14	– (90)	– (10.3)	3	– (30)	– (1.8)	1	– (10)	– (1.3)
Gloecercospora sorghi	47	100 (–)	21.5 (–)	14	70 (–)	7 (–)	6	40 (–)	6 (–)
Penicillium citrinum	2	10 (10)	0.5 (0.7)	4	40 (–)	2 (–)	3	20 (–)	3 (–)
Penicillium digitatum	4	30 (10)	1.4 (0.7)	8	40 (10)	3 (1.2)			
Penicillium wax	2	– (20)	– (1.5)	2	– (10)	– (1.2)	1	– (10)	– (1.3)
Phoma sorghina	25	100 (–)	11.4 (–)	18	50 (–)	9 (–)	2	10 (–)	2 (–)
Rhizopus stolonifer	10	10 (20)	1.4 (5.2)				2	20 (–)	2 (–)
Spadicoides obovata	19	70 (–)	8.7 (–)	3	20 (–)	1.5 (–)			
Trichoderma spp.	2	20 (–)	0.9 (–)	4	40 (–)	2 (–)	7	40 (10)	6 (1.3)
Total isolates	**355**			**367**			**230**		
Overall isolates	**889**								

Key: Values outside the brackets are data obtained from Blotter method and values in brackets are data obtained from PDA method

Table 8 Fungal species associated with sorghum grain mould in Metekel Zone by districts, Benishangul Gumuz, Ethiopia, during 2018 and 2019.

Fungal species	Bullen			Mandura			Pawi			Dangur			Guba		
	N	Freq%	RD%	N	Freq%	RD%	N	Freq%	RD%	N	Freq%	RD%	N	Freq%	RD%
Acremonium strictum				2	– (50)	– (10.5)	2	– (7.7)	– (2.2)				3	– (18.18)	– (1.7)
Alternaria alternata	3	– (60)	– (11.5)				18	38.5 (61.5)	5.1 (8.7)	6	10 (20)	3 (3.1)	22	63.6 (90.9)	6.2 (6.7)
A. brassicicola							4	7.7 (–)	2.03 (–)						
A. longipes							3	15.4 (–)	1.5 (–)						
A. tenuissima													1	9.09 (–)	0.62 (–)
Aspergillus flavus	2	20 (20)	1.5 (3.9)	6	100 (50)	14.3 (10.5)	6	23.08 (–)	3.1 (–)						
A. niger										4	– (20)	– (6.1)	4	– (36.36)	– (2.2)
Bipolaris sacchari							1	7.7 (–)	0.5 (–)						
Chaetomium oryzae							1	7.7 (–)	0.5 (–)						
Cladosporium sphaerospermum	5	80 (–)	7.4 (–)	1	50 (–)	3.6 (–)	56	92.3 (53.9)	22.8 (12)	31	100 (10)	22.4 (1.5)	27	100(18.2)	15.5 (1.12)
Colletotrichum graminicola							9	7.7 (30.8)	2.03 (5.4)	3	10 (10)	1.5 (1.5)	5	– (36.36)	– (2.8)
Curvularia australiensis													2	– (18.2)	– (1.12)
C. lunata	3	20 (20)	1.5 (7.7)				6	7.7 (30.8)	0.5 (5.4)	8	– (70)	– (12.1)	46	81.8 (81.8)	16.2 (11.2)
C. pallescens	1	– (20)	– (3.9)				5	15.4 (7.7)	2.03 (1.1)	3	– (30)	– (4.6)	4	– (27.27)	– (2.23)
Cylindrocarpon spp.	1	– (20)	– (3.9)	1	– (50)	– (5.3)	4	– (23.1)	– (4.4)	4	– (40)	– (6.1)	2	– (18.2)	– (1.12)
Epicoccum nigrum	11	80 (60)	8.8 (19.2)	7	100 (100)	10.7 (21.1)	13	38.5 (15.4)	3.6 (6.5)	1	10 (–)	0.8 (–)	67	36.4 (72.7)	3.1 (34.6)
Exserohilum rostratum							5	30.8 (–)	2.5 (–)	6	10 (20)	3 (3.03)	5	18.2 (27.3)	1.2 (1.7)
E. turcicum	14	100 (20)	19.1 (3.9)	3	100 (–)	10.7 (–)	43	100 (30.8)	18.8 (6.5)	32	100 (30)	21.6 (4.6)	51	100 (72.7)	23.6 (7.3)
Fusarium moniliforme	29	100 (60)	35.3 (19.2)	15	100 (100)	35.7 (26.3)	56	100 (92.3)	16.8 (25)	49	100 (100)	17.9 (37.9)	50	100 (90.9)	16.8 (12.9)
F. verticillioides							1	– (7.7)	1.1 (–)						
F. oxysporium	1	– (20)	3.9 (–)				2	– (15.4)	2.2 (–)	3	– (30)	– (4.6)	12	– (54.6)	– (6.7)

Table 8 Continued.

Fungal species	Bullen			Mandura			Pawi			Dangur			Guba		
	N	**Freq%**	**RD%**	**N**	**Freq%**	**RD%**	**N**	**Freq%**	**RD%**	**N**	**Freq%**	**RD%**	**N**	**Freq%**	**RD%**
F. semeticum										2	– (20)	– (3.03)	3	27.3 (–)	1.9 (–)
F. solani	2	– (20)	– (7.7)	4	– (100)	– (21.1)	10	– (53.9)	– (10.9)	4	– (40)	– (6.1)	6	– (36.4)	– (3.4)
Gloecercospora sorghi	3	40 (–)	4.4 (–)				12	69.2 (–)	6.1 (–)	29	100 (–)	21.6 (–)	13	81.8 (–)	8.1 (–)
Phoma sorghina	3	40 (–)	4.4 (–)				7	38.5 (–)	3.6 (–)	1	10 (–)	0.8 (–)	5	36.4 (–)	3.1 (–)
Penicillium citrinum		– (20)	– (3.9)				4	– (30.8)	– (4.4)						
P. digitatum	1			1	50 (–)	3.6 (–)	3	15.4 (–)	1.5 (–)				1	9.1 (–)	0.6 (–)
P. wax	3	– (20)	– (11.5)				2	– (15.4)	– (2.17)	3	– (20)	– (4.6)	4	– (27.3)	– (2.2)
Rhizopus stolonifer	4	40 (–)	5.9 (–)	1	– (50)	– (5.3)							4	18.2 (9.1)	1.9(0.6)
Spadicoides obovata							3	23.1 (–)	1.5 (–)	8	40 (–)	6 (–)	1	9.1 (–)	0.6 (–)
Trichoderma spp.	8	80 (–)	11.8 (–)	6	100 (–)	21.4 (–)	13	38.5 (15.4)	5.6 (2.2)	3	20 (10)	1.5 (1.5)	2	9.1 (9.1)	0.6 (0.6)
Total isolates	**94**			**47**			**289**			**200**			**340**		
Overall isolates	**970**														

Within the bracket PDA; Freq = Frequency; Rd = Relative density

Discussions

Results showed that all the sorghum grain samples collected from Assosa and Metekel Zones of Benishangul Gumuz, Ethiopia, were contaminated with various (a total of 32) grain mould fungal species categorized into 19 genera (Tables 4, 5). In other countries, grain mould fungal compositions of more than 40 genera had associated with sorghum grains (Navi et al. 1999, Astoreca et al. 2019) from the field (flowering) to storage levels (Thakur et al. 2003, Erpelding & Prom 2006, Alves dos Reis et al. 2010).

The most frequently and predominantly recovered mould fungal genera in the Assosa and Metekel zones were *Fusarium*, *Epicoccum*, *Exserohilum*, *Cladosporium*, *Curvularia*, *Gloecercospora*, *Phoma*, *Trichoderma*, *Alternaria*, and *Aspergillus*. Moreover, the predominant and frequently isolated mould fungi were *Fusarium moniliforme, Epicoccum nigrum*, *Exserohilum turcicum*, *Curvularia lunata*, *Alternaria alternata*, and *Cladosporium sphaerospermum*. Besides, *Fusarium semeticum* and *Phoma sorghina* were also detected with higher frequency from sorghum grains in the study area.

Our finding agrees with the studies that reported *Fusarium moniliforme*, *Fusarium semeticum*, *Culvularia lunata*, and *Phoma sorghina* as predominant species of mould fungi in other countries (Forbes et al. 1992, Singh & Agarwal 1993, Singh & Bandyopadhyay 2000, Muui et al. 2020). *Fusarium moniliforme*, *Fusarium semeticum*, *Culvularia lunata*, and *Phoma sorghina* had reported as the true grain mould pathogens that can infect immature sorghum grains. However, most recovered fungi were not true pathogens of grain mould but believed as superficial colonizers at later maturity stages or storages (Forbes et al. 1992, Singh & Agarwal 1993, Singh & Bandyopadhyay 2000).

Fusarium moniliforme is the major fungal pathogen that contaminate cereals like sorghum, maize, wheat, and rice (Kant et al. 2017). The pathogens adversely affect production through inhibiting sorghum seed germination (Vidhyasekaran 1983), causing diseases of seedling, stalk, root, head blight and grain mould that result in 30% yield losses (Lance 2022, Williams & Rao 1978). In addition, it causes stalk rot, top rot, and mouldy ears in maize (Osunlaja 1990). Besides, this pathogen can produce mycotoxins in contaminated sorghum grains that cause several human and animal diseases, including luekoencephalomalacia (LEM) in the horse, pulmonary edema in swine, and hepatoxicity in horses, swine, and rats.

Curvularia lunata was the other commonly recovered sorghum grain mould fungal species in the Assosa and Metekele Zones of Benishangul Gumuz region, Ethiopia. This pathogen was highly competent in humid (wet) conditions than *Fusarium* spp. and infected sorghum in early grain development stages (Little & Magill 2003, Prom et al. 2003). It badly lessens the viability of the seed (Prom et al. 2003, Prom 2004).

Even though, *Cladosporium*, *Epicoccum*, *Alternaria*, *Exserohilum*, and *Bipolaris* are considered as saprophytic invaders on sorghum, but have recovered from immature sorghum grains (Melake-Berhan et al. 1996). *Epicoccum nigrum* Llnk (synonym *E. purpurascens* Ehrenb.ex Schlecht) is a cosmopolitan mould fungus (Fávaro et al. 2011, Braga et al. 2018, D'Halewyn & Chevalier 2019). This pathogen had reported as one of the mould fungi responsible for causing mould in sorghum seed and sunflower seed in Serbia (Ristić et al. 2012, Milošević et al. 2019). Also, it has been reported as a seed pathogen of *Lotus* spp. that causing damping-off (Sisterna & Lori 2005) leaf spot (Colavolpe et al. 2018) to *Lotus* spp. in Argentina, maize leaf spot (Xu et al. 2022) in China. *Alternaria alternata* is among the hazardous fungi that cause toxins (*Alternaria* mycotoxins) which cause human oesophageal cancer (EFSA 2011, Tralamazza et al. 2018). Besides, *Alternaria* species in combination with *Cladosporium* species can cause sooty mould in mature grains (Li & Yoshizawa. 2000). *Exserohilum turcicum (*Pass.) Leonard & Suggs; synonym *Helminthosporium turcicum* Pass. is one of the dominant sorghum grain mould fungi in Benishangul Gumuz region, Ethiopia (Table 6). The pathogen causes leaf blight on sorghum, Maize, and Johnson grass (Agrios 2005, Ramathani et al. 2011).

Conclusion

In conclusion, *Fusarium moniliforme*, *Epicoccum nigrum*, *Exserohilum turcicum*, *Cladosporium sphaerospermum*, and *Gloecercospora sorghi* were most predominate fungal species followed by *Curvularia lunata*, *Alternaria alternate*, *Phoma sorghina*, *Aspergillus flavus*, *Colletotrichum graminicola*, and *Trichoderma* spp. *F. moniliforme*, *A. alternata*, and *A. flavus* are well known to produce potentially harmful toxins on several other cereals. Additional investigation on the amount of toxins contamination is required.

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