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Research Article

Identification of *Fusarium* Species Responsible to Cause Wheat Head Blight in Southwestern Ethiopia

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Abstract

Background and Objective: *Fusarium* Head Blight (FHB) caused by several *Fusarium* species is a dangerous disease of wheat and small cereals particularly in humid and sub-humid areas throughout the world. A loss due to FHB disease includes both grain yield and quality (that affect human and animal health). This investigation was aimed to identify FHB pathogens that cause blighted spikes in wheat across southwestern Ethiopia. **Materials and Methods:** A total of 269 single conidial isolates of *Fusarium* spp. were recovered from 52 FHB samples collected across South Western Ethiopia (SWE). Based on their colony, macroscopic and microscopic features, all the isolates were identified into nine species within the genus *Fusarium*. **Results:** Among the nine identified *Fusarium* species, *F. graminearum* and *F. culmorum* were most frequently recovered from blighted wheat spikes in southwestern Ethiopia. All the nine identified *Fusarium* species were pathogenic to a susceptible Danda'a wheat variety. Based on their AUDPC and spikelet infection severity, *F. avenaceum*, *F. poae*, *F. lateritium*, *F. culmorum*, *F. sambucinum*, *F. heterosporum* and *F. graminearum* were more aggressive ones that produced higher AUDPC ranging from 546.8-1067.2 and higher spikelet infection severity ranging from 57.8-100%. **Conclusion:** This study reveals the existence of *Fusarium* species diversity in Ethiopia that caused FHB on wheat. The pathogenic nature of all identified species indicated that FHB will become a potential disease on wheat in the area.

Key words: *Fusarium* head blight, *Fusarium* spp., pathogenicity, bread wheat, spikelet infection

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The necrotrophic *Fusarium* head blight (FHB) of wheat is a major head disease with an overwhelming impact on yield and grain quality mainly during wet seasons that favour FHB disease development and higher mycotoxin accumulation in grains^{1,2}. Generally, up to 19 species in the genus *Fusarium* were reported in causing FHB disease of wheat³. In addition to wheat, FHB disease infects several crops including barley, oats, rye, corn, canary seed, forage grasses, sugarcane and rice but wheat, barley and maize are the most affected crops⁴⁻⁷.

Kernel infection by FHB pathogens can cause poor seed germination, kernel shrivelling, reduction in the number of kernels per spike, low protein content and low baking quality that contributes to a significant loss both in yield and quality. Besides, the pathogen produces toxic metabolites that have health problems both to humans and animals when consumed⁸⁻¹¹.

Globally, due emphasis has been given for FHB because of its impact on grain yield as seedling blight, shrivelled kernels, infertility of spikes², grain quality such as low protein content, low baking quality¹², mycotoxin contaminations in grains and in straws which had health problems when feed by humans and animals^{8,9}.

However, there is a limited research effort done on the FHB disease of wheat in Ethiopia. One of the studies was reported a new novel species *F. aethiopicum* from 31 *Fusarium* cf. *graminearum* isolates originated from the Amhara and Oromia regions of Ethiopia¹³. This may indicate the existence of species diversity in the country. So far, all the past studies of FHB do not enclose Jimma, Buno-Bedele and West-Wollega zones where wheat is grown as one of the staple food crops. Therefore, this study has aimed to identify, characterize and test the pathogenicity of *Fusarium* spp. responsible for causing FHB disease of wheat in southwestern Ethiopia.

MATERIALS AND METHODS

Description of study areas: Blighted spikes of wheat were sampled at Dedo, Seka-Chekorsa districts of Jimma zone, Bedele and Gechi districts of the Buno-Bedele zone and Begi district of West-Wollega zone of Oromia region in Table 1.

Sample collection: Four wheat spikes with a typical FHB symptom were sampled per field and placed inside paper bags. The paper bags were labelled with sample code, date of sampling, altitude, latitude, longitude and collectors' names. The samples were placed in the icebox and also ventilated overnight to avoid excess water. Finally, the samples were taken to the Plant Pathology Laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) for isolation and identification of the causal agents.

Growth media: Malachite Green Agar (MGA) and Potato Dextrose Agar (PDA) were used to isolating *Fusarium* species from the samples. Spezieller Nährstoffarmer Agar (SNA) media with two sterile filter paper pieces had used to enhance the sporulation of isolates. Water agar (3% WA) media had been used for single conidial purification. Besides, PDA and Potato Sucrose Agar (PSA) had used for studying the colony characteristics (such as pigmentation and mycelial growth). All media used in this study had prepared according to 'The Fusarium Laboratory Manual'¹⁴. Also, all the media were amended by 250 mg of Chloramphenicol per litre of media to inhibit bacterial contaminants.

Isolation of *Fusarium* spp.: Eight kernels had separated from each blighted wheat spike sample and surface-sterilized in 4% (v/v) sodium hypochlorite solution for a minute, followed by thrice rinsing in sterilized distilled water. The kernels were well-drained under laminar flow. Then, four kernels had placed on the PDA plate and the other four kernels had placed on the

Table 1: Coordinates, elevations, annual rainfall and mean temperatures of the study area by districts, 2017

Zones	Districts	Coordinate		Altitude (m.a.s.l)	Rain fall (mm)	Temperature (°C)	
		N	E			Minimum	Maximum
Jimma	Dedo ^a	07°25'	37°00'	880-2800	1830.36	12.3	25.5
	Seka-Chekorsa ^c	07°35'	36°33'	1560-3000	1825.16	10.0	23.0
Buno-Bedele	Bede ^a	08°27'	36°21'	2012-2162	2051.1	13.0	26.4
	Gechi ^c	08°20'	36°40'	1400-2380	1639.0	18.0	25.0
West-Wollega	Begi ^b	09°15'	34°45'	1465-2100	1024.4	15.2	27.4

^aData obtained from National Meteorology Agency of Ethiopia, Jimma Meteorology Center, 2017, ^bData obtained from National Meteorology Agency of Ethiopia, Assosa Meteorology Center, 2017, Coordinate and altitude ranges were obtained from the respective district agriculture and natural resource development office, ^cData obtained from the respective district agriculture and natural resource development office

MGA plate. All plates were labelled, sealed with parafilm and incubated at 25°C. After 4-5 days of incubation, all *Fusarium* resembling colonies were cut along with the help of a sterile needle and transferred onto SNA plates. Both sides of the fungal agar block sterile filter paper pieces were placed to enhance conidia formation. The needle used was dipped in ethanol and burned off between each colony transfer. After the colony purification, all the Petri dishes were labelled, sealed with parafilm and incubated at 25°C for 7-17 days until sporulation.

Single conidium isolate development: For single conidial isolation, a small fungal plug was taken from sporulated SNA cultures and transferred to 3% WA and a drop of autoclaved distilled water was added onto the fungal plug and the conidia were dislodged by a sterile glass rod. The dislodged conidia were spread over the WA by a sterile glass rod spreader and the plates were incubated at 25°C for 24 hrs. Then, hyphal tips derived from a single conidium was cut and transferred to SNA with two sterile filter paper pieces¹⁴. The Petri dishes were then labelled, sealed with parafilm and incubated at 25°C for 7-17 days until sporulation. These isolates were used for the examination of microscopic and macroscopic features.

Identification of *Fusarium* spp.: Isolates of *Fusarium* recovered from blighted wheat spikes sampled across southwestern Ethiopia was identified into species level based on cultural and morphological characteristics as described by Leslie *et al.*¹⁴ and Refai *et al.*¹⁵.

Pathogenicity test

Experimental design and kernel disinfection: A pathogenicity experiment was conducted from February-June 2018 on Danda'a (a susceptible) bread wheat variety at JUCAVM, Jimma, Ethiopia. RCBD design with three replications had used. *Fusarium* species were used as test treatments, while sterile distilled water was used as a control. The experimental units were plastic pots (having a size of 15×11×15 cm), which had filled with an autoclaved potting mix (1:3:1 v/v sand/peat/compost). Before sowing, the wheat kernels had washed under running tap water for 5 min. Then, disinfected in 75% ethanol for 30 seconds and 0.5% NaOCl (sodium hypochlorite) solution for a minute. Finally, the kernels were rinsed twice in sterile distilled water and allowed to dry under laminar flow¹⁶. The four well-dried kernels had seeded at a depth of 2 cm in each pot. Each pot was fertilized with 5 g urea before emergence, 5 g NSP at tillering and 5 g urea at booting and also watered twice daily.

Preparation of inocula: The nine identified *Fusarium* spp. were recovered on SNA with sterile filter paper and incubated for 7-17 days at 25°C until sporulation. Then, 10 mL of sterilized distilled water was poured onto each sporulated plate and the conidia were dislodged by using a sterile glass road cell spreader. The suspension was filtered through two layers of sterilized cheesecloth¹⁶ and the final concentration was adjusted to 5×10^5 conidia mL⁻¹ with the help of a hemocytometer. From the adjusted inoculum, 200 µL of each *Fusarium* species was kept in a 5 mL Falcon tube at 4°C pending inoculation^{17,18}.

Inoculation: A single centrally positioned floret of two spikes per pot was injected¹⁹ at Zadok's growth stage 65 by the already prepared 10 µL inoculum of each *Fusarium* species. Control (check) spikes were inoculated in the same way by 10 µL of sterile distilled water. Simultaneously, the spikes were tagged and covered with polythene bags for 48 hrs to maintain high humidity that can facilitate infection process²¹⁻²³.

Collected data

***Fusarium* morphology data:** Primary and secondary morphology data were collected for identification of *Fusarium* isolates into species level according to the description of *Fusarium* species described by Leslie *et al.*¹⁴, He *et al.*²⁰ and Refai *et al.*¹⁵.

Primary characters such as:

- Macroconidia characteristics like phialides, shape, size, number of septa, the shape of the apical and basal cells were noted
- Microconidia characteristics including presence or absence of microconidia, if present their shape, size and how they are formed (phialides) were noted
- Chlamydospores presence or absence, if present their form (chain or single)

Secondary characters:

- Colony morphology features include colour on PDA, pigmentation and hyphal colony growth on PDA and PSA

Pathogenicity test data: Blighted spikelets per spike due to the infection of inoculated *Fusarium* spp. was carefully inspected every week. The spikelet bleaching severity caused by each *Fusarium* spp. was recorded as a percentage of blighted spikelets over the total number of spikelets per spike²⁴ at 7, 14, 21 and 28 days after inoculation^{17,20,25}. Finally,

each inoculated spike was separately taken to the laboratory and re-isolation was performed to confirm the identity of the test pathogen.

Data analysis: From the pathogenicity test experiment, the Area Under Disease Progress Curve (AUDPC) for the nine *Fusarium* spp. was determined as described by Madden *et al.*²⁶:

$$AUDPC = \sum_{i=7}^n \left\{ \left(\frac{y_i + y_{i+7}}{2} \right) (t_i - t_{i-7}) \right\}$$

Where:

AUDPC = Area under disease progress curve

n = Total number of observation days at the *i*th observation

y_i = Spikelet bleaching severity at the *i*th observation

t = Time at the *i*th observation

Analysis of variance for spikelet bleaching severity and AUDPC data was performed using the general linear model procedure of SAS version 9.3 statistical software²⁷. The means were separated by the LSD test at a probability level of 0.05. The spikelet infection rates of each inoculated species were determined by Minitab 17 software. The RCBD model used for analyzing AUDPC and spikelet bleaching severity is described as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where:

Y_{ij} = Response (AUDPC or spikelet bleaching severity) for treatment *i* observed in block *j*

μ = Overall mean

α = Effect of the *i*th treatment

β = Effect of the *j*th block

ε_{ij} = Error term for the *i*th treatment in the *j*th block

Finally, the aggressiveness of *Fusarium* spp. used in pathogenicity test on Danda'a wheat variety was determined from spikelet infection severity and AUDPC^{28,29}.

RESULTS AND DISCUSSION

Fusarium spp. associated with blighted wheat spikes: A

total of 269 single conidial purified *Fusarium* isolates had recovered from blighted wheat spikes collected during the 2017 main cropping season in Jimma, Buno-Bedele and West-Wollega zones of Oromia, Southwestern Ethiopia. Based on their cultural and microscopic characteristics as described by Leslie *et al.*¹⁴ and Refai *et al.*¹⁵, all the identified isolates were grouped into nine *Fusarium* species (Fig. 1-9a-c) with varied isolation frequency across the study area in Table 2. The variation may be due to factors such as field location, climatic conditions, soil management, crop rotation and cultivation methods³⁰.

Based on this provisional identification, *F. culmorum* (Fig. 2a-c) and *F. ussuriarum* (Fig. 5a-c) were isolated from blighted wheat spikes which were not reported by the previous study conducted in Ethiopia, though this needs further confirmation. On the other hand, *F. graminearum*, *F. avenaceum*, *F. poae*, *F. semitectum*, *F. sambucinum*, *F. heterosporum* and *F. lateritium* had recovered from stored wheat grains and blighted wheat spikes sampled from Arsi, Bale, Gojam, Gonder, Shoa and Wollo areas.

Among the nine species, *F. graminearum* and *F. culmorum* were the two most frequently isolated species comprised of 29.0 and 26.4% of the total number of *Fusarium* isolates, respectively. Whereas, *F. avenaceum*, *F. poae*, *F. ussuriarum*, *F. semitectum*, *F. sambucinum* and *F. lateritium* had made up of 10.4, 7.4, 6.7, 6.3, 6.0 and 6.0%, respectively. On the other hand, the least isolated species was *F. heterosporum* which had only 1.9% of the total isolates (Table 2). These revealed that *F. graminearum* and

Table 2: Isolation frequency (%) of identified *Fusarium* spp. from wheat blighted heads in SWE, 2017 main cropping season

<i>Fusarium</i> species	N	PDA	N	MGA	TN	TIF
<i>F. graminearum</i> Schwabe (Fig. 1a-c)	46	28.6	32	29.6	78	29.0
<i>F. culmorum</i> (W.G. Smith) Saccardo (Fig. 2a-c)	32	19.9	39	36.1	71	26.4
<i>F. avenaceum</i> (Fries) Saccardo (Fig. 3a-c)	21	13.0	7	6.5	28	10.4
<i>F. poae</i> (Peck) Wollenweber (Fig. 4a-c)	12	7.5	8	7.4	20	7.4
<i>F. ussuriarum</i> T. Aoki, Gagkaeva, Yli-Mattila, Kistler and O'Donnell (Fig. 5a-c)	12	7.5	6	5.6	18	6.7
<i>F. semitectum</i> Berkeley and Ravenel (Fig. 6a-c)	12	7.5	5	4.6	17	6.3
<i>F. sambucinum</i> Fückel <i>sensu stricto</i> (Fig. 7a-c)	10	6.2	6	5.6	16	6.0
<i>F. lateritium</i> Nees (Fig. 8a-c)	13	8.1	3	2.8	16	6.0
<i>F. heterosporum</i> Nees <i>ex</i> Fries (Fig. 9a-c)	3	1.9	2	1.9	5	1.9
Total	161		108		269	

N: Number of isolates, PDA: % of isolates on potato dextrose agar, MGA: % of isolates on Malachite-Green Agar; TN: Total number frequency (%), TIF: Total isolation frequency (%)

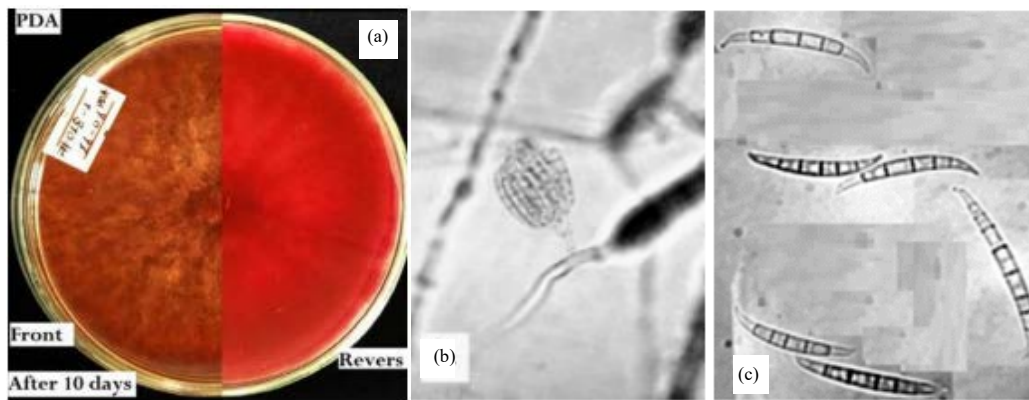


Fig. 1(a-c): *F. graminearum* colony on PDA, (b) Conidiophore of *F. graminearum* and (c) Conidia of *F. graminearum*

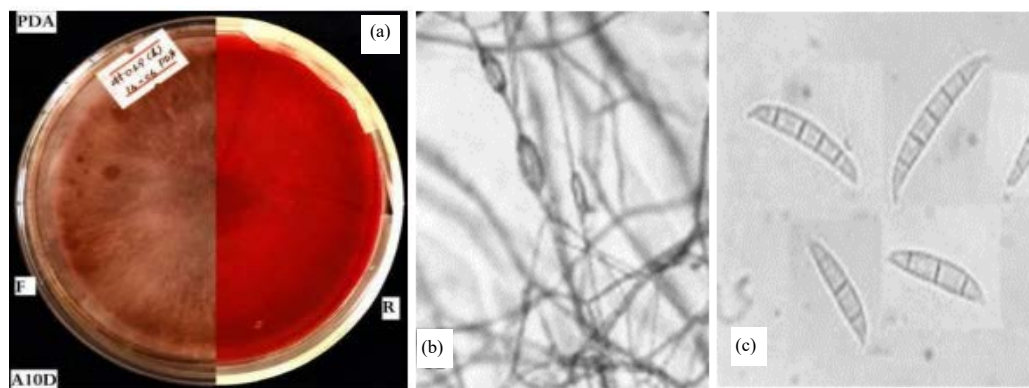


Fig. 2(a-c): *F. culmorum* colony on PDA, (b) Conidiophore of *F. culmorum* and (c) Conidia of *F. culmorum*

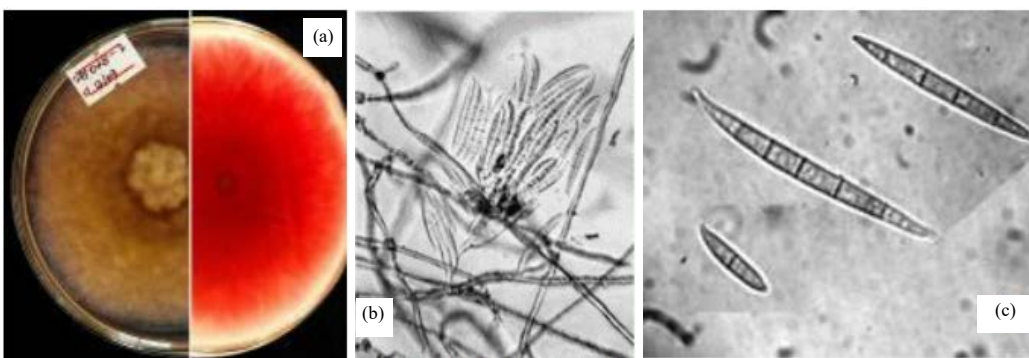


Fig. 3(a-c): *F. avenaceum* colony on PDA, (b) Conidiophore of *F. avenaceum* and (c) Conidia of *F. avenaceum*

F. culmorum were the two most predominately isolated species followed by *F. avenaceum* from blighted wheat spikes in southwestern Ethiopia. Previously in Ethiopia, *F. graminearum* and *F. avenaceum* were reported among the predominant species isolated from stored wheat grains

and blighted wheat spikes sampled from Arsi, Bale, Gojam, Gonder, Shoa and Wollo areas. Further, in neighbouring country Kenya, these two species (*F. graminearum* and *F. avenaceum*) were predominately isolated from wheat spikes in Narok County and kernels in Nakuru County³¹.

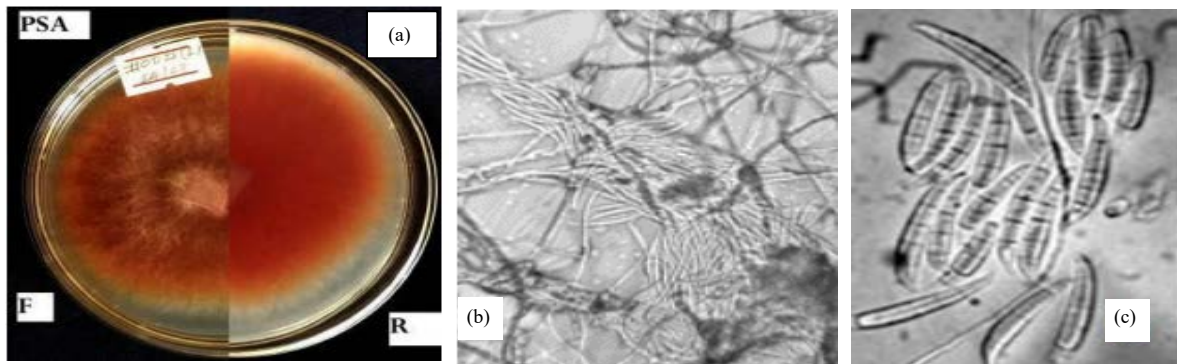


Fig. 4(a-c): *F. lateritium* colony on PSA, (b) Conidiophore of *F. lateritium* and (c) Conidia of *F. lateritium*

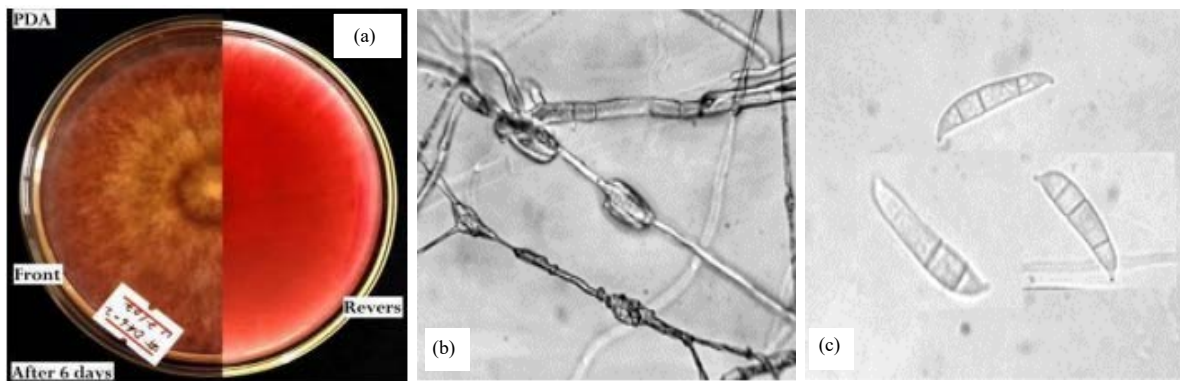


Fig. 5(a-c): *F. poae* colony on PDA, (b) Conidiophore of *F. poae* and (c) Conidia of *F. poae*

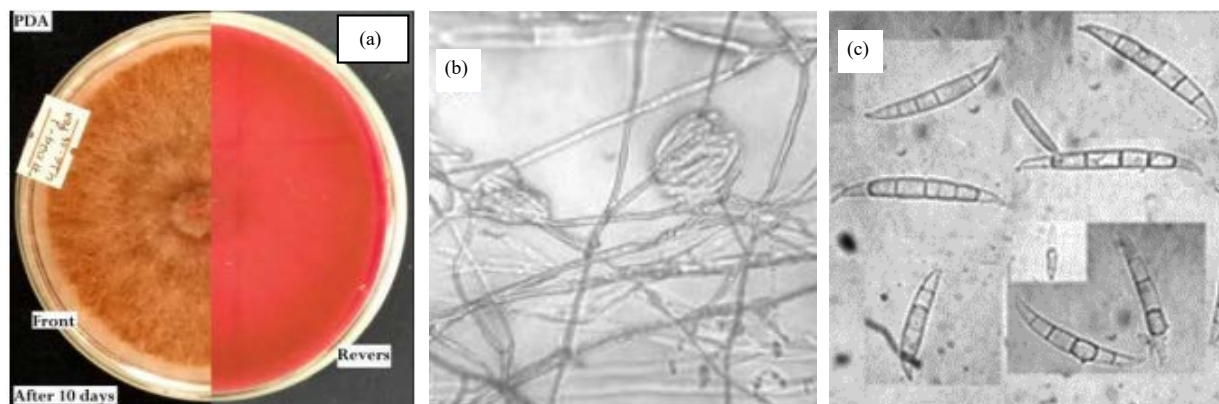


Fig. 6(a-c): *F. semitectum* colony on PDA, (b) Conidiophore of *F. semitectum* and (c) Conidia of *F. semitectum*

Distribution of *Fusarium* spp. in southwestern Ethiopia:

F. graminearum, *F. culmorum*, *F. lateritium*, *F. avenaceum*, *F. poae* and *F. heterosporum* were isolated from samples collected from the five assessed districts in southwestern Ethiopia in Table 3. However, *F. sambucinum*, *F. ussuriarum*

and *F. semitectum* were isolated from four assessed districts (Table 3).

The most dominant *F. graminearum* was mainly isolated from samples of the Buno-Bedele zone (44.9%) and West-Wollega zone (34.6%). In particular, Begi, Bedele and

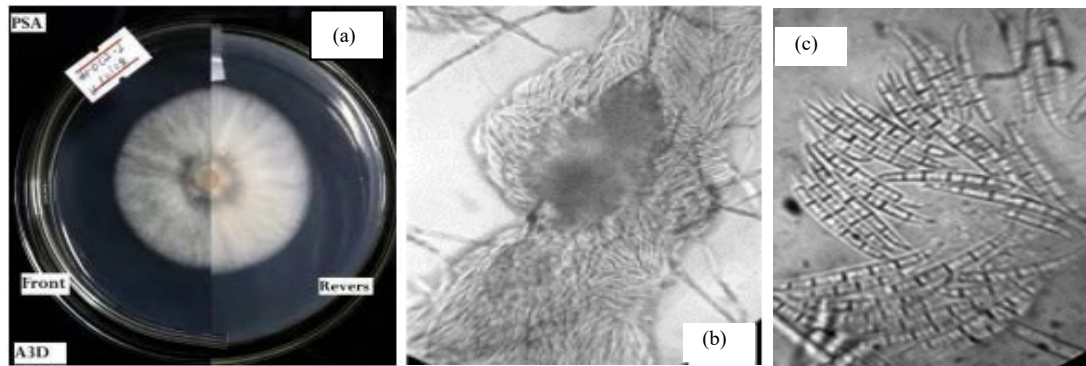


Fig. 7a: *F. ussurianum* colony on PSA, Fig. 7b: Conidiophore of *F. ussurianum*, Fig. 7c: Conidia of *F. ussurianum*

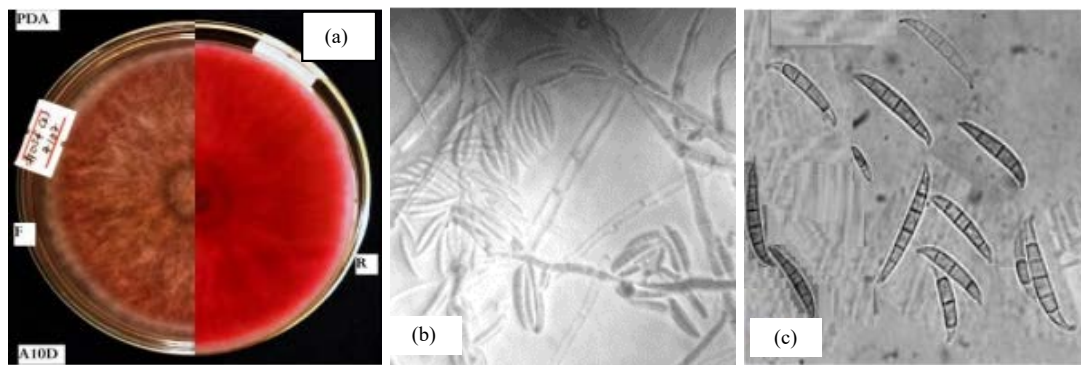


Fig. 8a: *F. sambucinum* colony on PDA, Fig. 8b: Conidiophore of *F. sambucinum*, Fig. 8c: Conidia of *F. sambucinum*

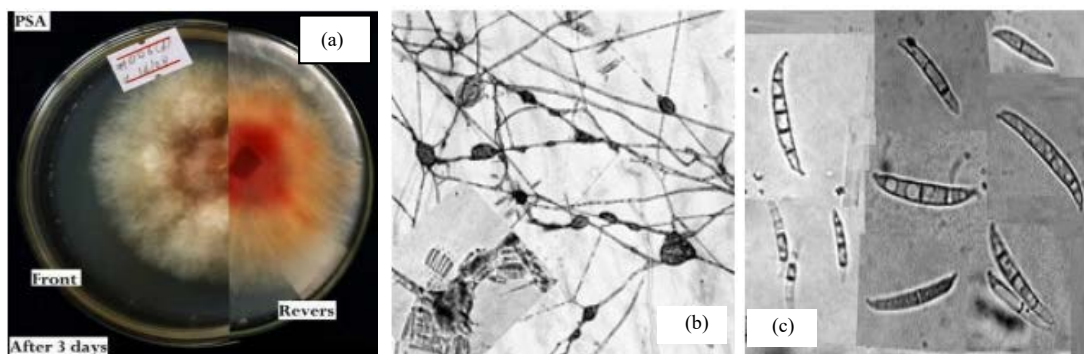


Fig. 9a: *F. heterosporum* colony on PSA, Fig. 9b: Conidiophore of *F. heterosporum*, Fig. 9c: Conidia of *F. heterosporum*

Gechi districts were attributed 24.4, 23.1 and 21.8% of *F. graminearum* isolation, respectively (Table 3). Whereas, the second predominant *F. culmorum* was frequently isolated from samples of the Buno-Bedele zone (59.2%) and Jimma zone (25.4%). It was mainly recovered from samples of Gechi district (39.4%), Bedele district (19.7%) and Seka-Chekorsa (19.7%) (Table 3). The third predominant *F. avenaceum* was

mainly isolated from samples of Jimma zone (53.6%) particularly in Seka-Chekorsa that attributed 38.5% isolation frequency (Table 3).

The occurrence and distribution of *Fusarium* species can vary with the changing climate, crop rotation, cultivar resistance and interactions among different species^{30,32}. For instance, in some parts of Europe, the predominant species

Table 3: Distribution (%) of *Fusarium* spp. by zones and districts in southwestern, 2017

<i>Fusarium</i> spp.	N	Distribution by zones			Distribution by districts				
		Jimma	Buno-Bede	West-Wollega	Dedo	Seka-Chekorsa	Bede	Gechi	Begi
<i>F. graminearum</i>	78	16 (20.5)	35 (44.9)	27 (34.6)	8 (10.3)	8 (10.3)	18 (23.1)	17 (21.8)	19 (24.4)
<i>F. culmorum</i>	71	18 (25.4)	42 (59.2)	11 (15.5)	4 (5.6)	14 (19.7)	14 (19.7)	28 (39.4)	8 (11.3)
<i>F. lateritium</i>	16	6 (37.5)	6 (37.5)	4 (25.0)	1 (6.3)	5 (31.3)	3 (18.8)	3 (18.8)	3 (18.8)
<i>F. avenaceum</i>	28	15 (53.6)	7 (25.0)	6 (21.4)	5 (19.2)	10 (38.5)	2 (7.7)	5 (19.2)	4 (15.4)
<i>F. poae</i>	20	5 (25.0)	6 (30.0)	9 (45.0)	3 (15.0)	2 (10.0)	3 (15.00)	3 (15.0)	5 (25.0)
<i>F. sambucinum</i>	16	4 (25.0)	8 (50.0)	4 (25.0)	4 (25.0)	-	3 (18.8)	5 (31.3)	1 (6.3)
<i>F. ussuriarum</i>	18	9 (50.0)	9 (50.0)	-	4 (22.2)	5 (27.8)	6 (33.3)	3 (16.7)	-
<i>F. semitectum</i>	17	9 (52.9)	5 (29.4)	3 (17.7)	4 (23.5)	5 (29.4)	-	5 (29.4)	2 (11.8)
<i>F. heterosporum</i>	5	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)

Values in parenthesis are percent frequency; -: Shows the species does not recover from the samples

Table 4: Blighted spikelet severity and AUDPC of *Fusarium* spp. under lath-house, 2018

<i>Fusarium</i> spp.	Spikelet infection severity				AUDPC	r	R ² (%)
	7 DAI	14 DAI	21 DAI	28 DAI			
<i>F. avenaceum</i>	2.6 ^{cd}	30.5 ^{ab}	70.7 ^a	100.0 ^a	1067.2 ^a	0.51**	64.62
<i>F. poae</i>	9.6 ^a	35.9 ^a	74.4 ^a	74.5 ^{ab}	1066.3 ^a	0.52**	85.58
<i>F. sambucinum</i>	6.1 ^{abc}	16.3 ^{abcd}	52.3 ^{abc}	83.1 ^a	792.4 ^{ab}	0.11	4.50
<i>F. lateritium</i>	5.5 ^{bc}	21.9 ^{abc}	54.9 ^{ab}	85.6 ^a	856.2 ^{ab}	0.43**	70.45
<i>F. culmorum</i>	6.0 ^{abc}	20.3 ^{abcd}	46.7 ^{bc}	88.9 ^a	801.3 ^{ab}	0.52*	44.51
<i>F. heterosporum</i>	6.4 ^{ab}	22.9 ^{abc}	40.9 ^{bc}	57.8 ^{ab}	670.9 ^b	0.26*	45.29
<i>F. graminearum</i>	4.9 ^{bc}	13.2 ^{bcd}	29.1 ^{cd}	66.8 ^{ab}	546.8 ^b	0.21**	43.19
<i>F. ussuriarum</i>	0.0 ^d	3.0 ^{cd}	7.1 ^{de}	29.8 ^{cd}	175.2 ^c	0.42**	60.36
<i>F. semitectum</i>	0.0 ^d	0.0 ^d	0.0 ^e	33.2 ^{bc}	116.2 ^c	0.12	5.58
Sterilized distilled water	0.00 ^d	0.0 ^d	0.0 ^e	0.0 ^c	0.0 ^c	-	-
LSD	3.8	20.4	23.7	45.8	358.7		

Mean values in a column with different letters are significant at $p < 0.05$, AUDPC: Area under disease progress curve, DAI: Days after inoculation, LSD: Least significant difference, r: Rate of spikelet bleaching

were varied among *F. graminearum*, *F. poae*, *F. avenaceum* and *F. culmorum*³², however, *F. graminearum* was also reported in displacing the *F. culmorum*³³. Moreover, a four-year study in Belgium revealed that the most frequent causal agent of FHB in wheat was *F. graminearum* mainly in areas where corn was cultivated and *F. culmorum*, mainly in areas where small grains were grown³⁴. This revealed the effect of cultural practices on *Fusarium* species abundance.

Pathogenicity test: The pathogenicity of all *Fusarium* spp. identified in this study was assessed using point (single spikelet) injection method¹⁸. The results indicated that all the tested *Fusarium* spp. caused FHB symptoms on spikes of Danda'a variety. However, no FHB symptoms were observed on spikes inoculated with sterile distilled water (control). Re-isolation from the kernels of inoculated spikes agrees with descriptions of the inoculated species, which confirms their pathogenicity under Lath-house conditions.

Fusarium spp. had shown significantly varied spikelet bleaching severity and AUDPC on Danda'a wheat variety in Table 4. *F. avenaceum* was the most aggressive species that caused the highest spikelet bleaching severity of 100% at 28 Days After Inoculation (DAI) and AUDPC of 1067.2 on

Danda'a variety (Table 4). Statistically comparable spikelet bleaching severities had produced by *F. culmorum*, *F. graminearum*, *F. lateritium*, *F. sambucinum*, *F. poae* and *F. heterosporum*. Likewise, *F. poae*, *F. sambucinum*, *F. lateritium* and *F. culmorum* were generated statistically similar AUDPC as compared to that of *F. avenaceum*. However, *F. ussuriarum* and *F. semitectum* had produced the lower AUDPC of 175.2 and 116.2, respectively (Table 4).

All nine *Fusarium* species had shown the different rates of FHB disease development on Danda'a wheat variety (Table 4). Seven of the tested species had caused FHB symptoms at 7 DAI, while the others at 14 and 28 DAI (Table 4). This finding almost agrees with the comparative aggressiveness study conducted in Canada that reported *F. graminearum*, *F. avenaceum*, *F. culmorum* and *F. poae* had produced visible spikelet bleaching at 21 and 28 DAI on wheat spikes³⁵. On the other hand, delayed symptom development was observed by *F. ussuriarum* and *F. semitectum* after seven and 21 DAI (Table 4).

Based on spikelet bleaching severity and AUDPC results, *F. avenaceum*, *F. poae*, *F. sambucinum*, *F. lateritium*, *F. culmorum*, *F. heterosporum* and *F. graminearum* were more aggressive on Danda'a wheat variety. These seven

species had caused spikelet bleaching severity and AUDPC beyond or equal to 57.8 and 546.8%, respectively (Table 4). Whereas, *F. semitectum* and *F. ussuriarum* were showed less aggressiveness on Danda'a variety with spikelet bleaching severity of 33.19 and 29.78% and AUDPC of 116.2 and 175.2, respectively (Table 4). These findings concurred with the aggressiveness study that reported *F. graminearum* and *F. culmorum* as an aggressive species causing more than 35% of spikelet bleaching severity of wheat in Canada³⁵.

In addition to causing blighted wheat spikes, *F. culmorum*, *F. graminearum* and *F. avenaceum* had responsible for crown rot of bread wheat and durum wheat in Turkey³⁶ and root rot of corn, soybean and wheat in Nebraska³⁷. Likewise, *F. culmorum* had been reported in causing higher seedling blight, while *F. graminearum* had responsible for causing severe crown rot of wheat^{38,39}.

CONCLUSION

A total of 269 single conidial purified isolates had recovered from blighted wheat spikes sampled across Jimma, Buno-Bede and West-Welega zones of Oromia, southwestern Ethiopia. Based on their cultural and microscopical characteristics, all isolates had classified into nine *Fusarium* species. Among the nine species, *F. graminearum* and *F. culmorum* were the most predominant ones, followed by *F. avenaceum* in southwestern Ethiopia. Besides, all the nine species had pathogenic and *F. avenaceum*, *F. poae*, *F. sambucinum*, *F. lateritium*, *F. culmorum*, *F. heterosporum* and *F. graminearum* were shown more aggressiveness on Danda'a wheat variety.

SIGNIFICANCE STATEMENT

This study discovered nine pathogenic *Fusarium* species responsible for the FHB of wheat in southwestern Ethiopia. This study noticed two *Fusarium* species namely *F. culmorum* and *F. ussuriarum* that were not reported in Ethiopia. Besides, this investigation identified *F. graminearum* and *F. culmorum* as the most frequent and the more aggressive species that caused FHB on wheat in the study area. Therefore, this study will help the researchers to uncover why these species are dominant in the area and also help researchers to devise intervention strategies.

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