

DOI: 10.36648/plantpathology.3.1.04

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

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Received date: May 25, 2020; **Accepted date:** June 08, 2020; **Published date:** June 15, 2020

Citation: Kebede M, Adugna G, Hundie B (2020) Identification of *Fusarium* Species Responsible to Cause Wheat Head Blight in Southwestern Ethiopia. Res J Plant Pathol Vol. 3 No.1:4.

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Abstract

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. Losses 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. A total of 269 single conidial isolates of *Fusarium* spp. were recovered from 52 FHB samples collected across southwestern Ethiopia. Based on their colony, macroscopic and microscopic features, all the isolates were identified into 9 species within the genus *Fusarium*. Among them, *F. graminearum* (29.39%) and *F. culmorum* (26.41%) were the dominant species followed by *F. avenaceum* (10.4%), *F. poae* (7.4%), *F. ussurianum* (6.7%), *F. semitectum* (6.3%), *F. lateritium* (6.0%), *F. sambucinum* (6.0%) and *F. heterosporum* (1.9%) in southwestern Ethiopia. The pathogenicity test revealed that all the 9 identified *Fusarium* species were caused typical FHB symptoms on spikes of 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 to 1067.2 and higher spikelet infection severity ranging from 57.8% to 100%.

[1,2]. Generally, up to 19 species in the genus *Fusarium* were reported in causing FHB disease of wheat [3]. 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 crops [4-7].

Kernel infection by FHB pathogens can cause poor seed germination, kernel shriveling, reduction in the number of kernels per spike, low protein content, and low baking quality that contribute 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 consumed [8-11].

Globally, due emphasize has given for FHB because of its impact on grain yield as seedling blight, shriveled kernels, and infertility of spikes [1], grain quality such as low protein content, and low baking quality [12], and mycotoxin contaminations in grains, and in straws which had health problem when feed by humans and animals [8,9].

However, there is a limited research effort done on FHB disease of wheat in Ethiopia. One of the efforts was, yield loss of 60% and more were recorded on wheat under natural infection at Holeta [13]. The other was, identification of 17 and 13 *Fusarium* species from stored wheat grains, and blighted wheat spikes across Arsi, Bale, Gojam, Gonder, Shoa, and Wollo areas [14], but their pathogenicity tests were not verified. Additionally, a new novel species *F. aethiopicum* has phylogenetically identified from 31 *Fusarium cf. graminearum* isolates originated from Amhara and Oromia regions of Ethiopia [15]. 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-Wellega zones where wheat is grown as one of the staple food crops. Therefore, this study had aimed to identify, characterize, and test the pathogenicity of *Fusarium* spp. responsible for causing FHB disease of wheat in southwestern Ethiopia.

Keywords: *Fusarium* head blight; FHB; *Fusarium*spp; Pathogenicity; Bread wheat

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 favor FHB disease development, and higher mycotoxin accumulation in grains

Materials and Methods

Description of study areas

Blighted spikes of wheat were sampled at Dedo, and Seka-Chekorssa districts of Jimma zone, Bedele and Gechi districts of

the Buno-Bedele zone, and Begi district of West-Wollega zone of Oromia region (Table 1).

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			Min.	Max.
Jimma	Dedoa	07° 25'	37° 00'	880-2800	1830.36	12.3	25.5
	Seka-Chekorssac	07° 35'	36° 33'	1560-3000	1825.16	10	23
Buno-Bedele	Bedelea	08° 27'	36° 21'	2012-2162	2051.1	13	26.4
	Gechic	08° 20'	36° 40'	1400-2380	1639	18	25
West-Wollega	Begib	09° 15'	34° 45'	1465-2100	1024.4	15.2	27.4

a. Data obtained from National Meteorology Agency of Ethiopia, Jimma Meteorology Center, 2017

b. Data 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.

c. Data obtained from the respective district agriculture and natural resource development office.

Sample collection

Four wheat spikes with a typical FHB symptom were sampled per field and placed inside paper bags. The paper bags were labeled 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 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' and '*Fusarium* species: an illustrated manual for identification' [16,17]. Also, all the media were amended by 250 mg of Chloramphenicol per liter of media to inhibit bacterial contaminants.

Isolation of *Fusarium* spp.

Eight kernels had separated from each blighted wheat spike samples 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 PDA plate and the other four kernels had placed on MGA plate. All plates were labeled, sealed with parafilm and incubated at 25°C. After 4 to 5 days of incubation, all *Fusarium* resembling colonies were cut along with the help of sterile needle and transferred onto SNA plates. Both side 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 labeled, sealed with parafilm and incubated at 25°C for 7 to 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 sterile glass road. The dislodged conidia were spread over the WA by sterile glass road spreader and the plates were incubated at 25°C for 24 hours. Then after, a hyphal tip derived from a single conidium was cut and transferred to SNA with two sterile filter paper pieces [17]. The Petri dishes were then labeled, sealed with parafilm and incubated at 25°C for 7 to 17 days until sporulation. These isolates were used for examination of microscopic and macroscopic features.

Identification of *Fusarium* spp.

Isolates of *Fusarium* recovered from blighted wheat spikes sampled across southwestern Ethiopia was identified in to species level based on cultural and morphological characteristics as described by [16-18].

Pathogenicity test

Experimental design and kernel disinfection: Pathogenicity experiment was conducted from February 2018 to 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 cm × 11 cm × 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 five minutes. 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 flow [19]. 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 sterile glass road cell spreader. The suspension was filtered through two layers of sterilized cheesecloth [19] and the final concentration was adjusted to 5 × 10⁵ conidia ml⁻¹ with the help of hemocytometer. From the adjusted inoculum, 200 μl of each *Fusarium* species was kept in a 5 ml Falcon tube at 4°C pending for inoculation [20,21].

Inoculation: A single centrally positioned floret of two spikes per pot were injected [22] at Zadok's growth stage 65 [23] 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 hours to maintain high humidity that can facilitate infection process [24-26].

Collected data

***Fusarium* morphology data:** Primary and secondary morphology data were collected for identification *Fusarium* isolates in to species level according to the description of species described by [16-18]. The primary characters such as a) Macroconidia characteristics like phialides, shape, size, number of septa, shape of the apical and basal cells was noted; b) Microconidia characteristics including presence or absence of microconidia, if present their shape, size and the manner in which they are formed (phialides) were noted; and c) Chlamydospores presence or absence, if present their form (chain or single). Secondary characters; a) Colony morphology features that includes color on PDA, pigmentation and hyphal colony growth on PDA and PSA.

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	46	28.6	32	29.6	78	29

Pathogenicity test data: Blighted spikelets per spikes due to the infection of inoculated *Fusarium* spp. was carefully inspected at weekly basis. The spikelet bleaching severity caused by each *Fusarium* spp. was recorded as a percentage of blighted spikelets over the total number of spikelets per spike [28] at 7, 14, 21 and 28 days after inoculation [29]. Finally, each inoculated spike was separately taken to 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 [30].

Where; AUDPC is the area under disease progress curve, n is total number of observation days at the *i*th observation, *y_i* is spikelet bleaching severity at the *i*th observation, *t* is 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 software [31]. The means were separated by LSD test at a probability level of 0.05. The spikelet infection rate of each inoculated species was determined by Minitab 17 software. The RCBD model used for analyzing AUDPC and spikelet bleaching severity is described as follows:

Where; *Y_{ij}* is the response (AUDPC or spikelet bleaching severity) for treatment *i* observed in block *j*; *μ* is the overall mean, *α* is the effect of the *i*th treatment, *β* is the effect of the *j*th block, *ε_{ij}* is the error term for the *i*th treatment in the *j*th block.

Finally, aggressiveness of *Fusarium* spp. used in pathogenicity test on Danda'a wheat variety was determined from spikelet infection severity and AUDPC [32,33].

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 [16-18], all isolates were grouped into nine *Fusarium* species (**Figure 1**) with varied isolation frequency across the study area (**Table 2**). The variation may be due to factors such as field location, climatic conditions, soil management, crop rotation and cultivation methods [34].

<i>F. culmorum</i> (W.G. Smith) Saccardo	32	19.9	39	36.1	71	26.4
<i>F. avenaceum</i> (Fries) Saccardo	21	13	7	6.5	28	10.4
<i>F. poae</i> (Peck) Wollenweber	12	7.5	8	7.4	20	7.4
<i>F. ussurianum</i> T. Aoki, Gagkaeva, Yli-Mattila, Kistler and O'Donnell	12	7.5	6	5.6	18	6.7
<i>F. semitectum</i> Berkeley and Ravenel	12	7.5	5	4.6	17	6.3
<i>F. sambucinum</i> Fückelsensustricto	10	6.2	6	5.6	16	6
<i>F. lateritium</i> Nees	13	8.1	3	2.8	16	6
<i>F. heterosporum</i> Neesex Fries	3	1.9	2	1.9	5	1.9
Total	161		108		269	

Based on this provisional identification, *F. culmorum* and *F. ussurianum* 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 [14].

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. ussurianum*, *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 *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 [14].

Further, in neighboring country Kenya, these two species (*F. graminearum* and *F. avenaceum*) were predominately isolated from wheat spikes in Narok County, and kernels in Nakuru County [35].

N: Number of Isolates; PDA: % of Isolates on Potato Dextrose Agar; MGA: % of Isolates on Malchet-Green Agar; TN: Total Isolation Frequency (%); TIF: Total Isolation Frequency (%)

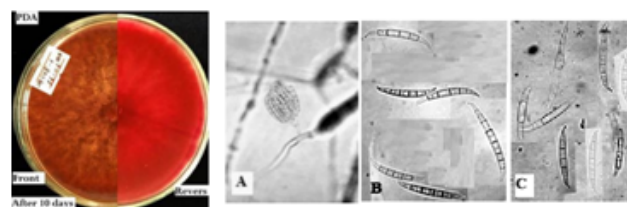


Figure 1: *F. graminearum*; A: conidiophore, and B: and amp; C: conidia

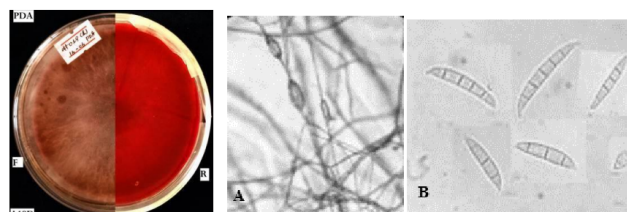


Figure 2: *F. culmorum*; A: conidiophore, and B: conidia

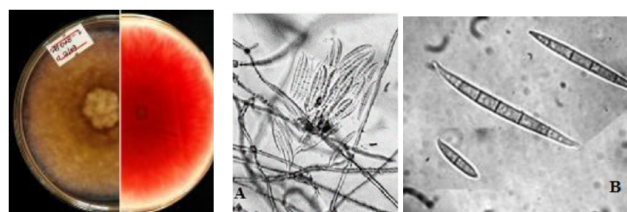


Figure 3: *F. avenaceum*; A: conidiophore, and B: conidia

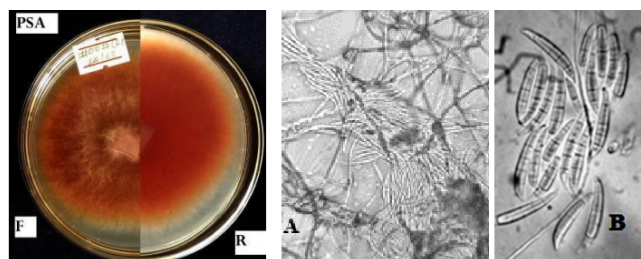


Figure 4: *F. lateritium*; A: conidiophore, and B: conidia

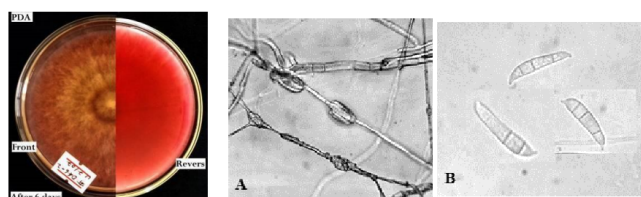


Figure 5: *F. poae*; A: conidiophore, and B: conidia

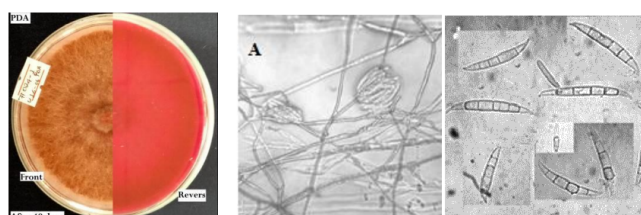


Figure 6: *F. semitectum* A: conidiophore, and B: conidia

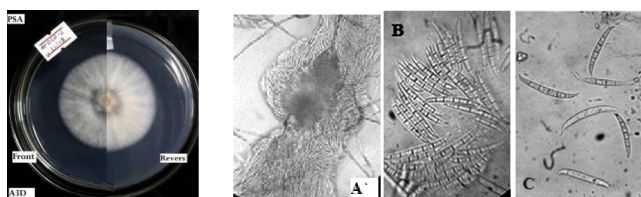


Figure 7: *F. ussurianum*; A: conidiophore, and B and C: conidia

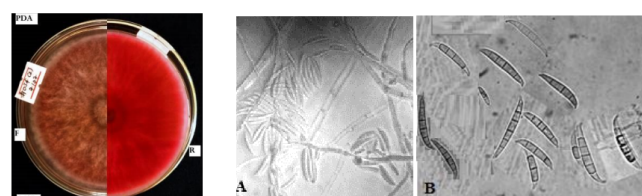


Figure 8: *F. sambucinum*; A: conidiophore and B: conidia

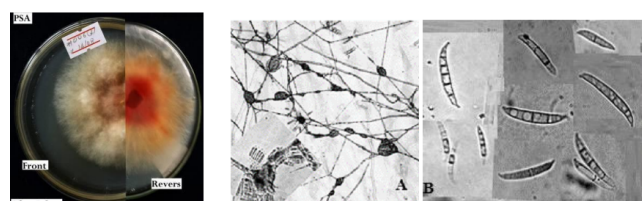


Figure 9: *F. heterosporum*; A: conidiophore, and B: conidia

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 (Table 3). However, *F. sambucinum*, *F. ussurianum*, and *F. semitectum* were isolated from four assessed districts.

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 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-Chekorssa (19.7%).

The third predominant *F. avenaceum* was mainly isolated from samples of Jimma zone (53.6%) particularly in Seka-Chekorssa that attributed 38.5% isolation frequency (Figure 2).

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-Bedele	West-Wollega	Dedo	Seka-Chekorssa	Bedele	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 is percent frequency; -shows the specie does not recovered from the samples.

The occurrence and distribution of *Fusarium* species can vary with the changing climate, crop rotation, cultivar resistance and interactions among different species [34,36]. For instance, in some parts of Europe, the predominant species were varied among *F. graminearum*, *F. poae*, *F. avenaceum* and *F. culmorum* [36], however, *F. graminearum* was also reported in displacing the *F. culmorum* [37]. 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 grown (Figure 3) [38]. This clearly 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 method (Figure 4) [22]. The results indicated that all the tested *Fusarium* spp. caused FHB symptoms on spikes of Danda'a variety (Figure 5). 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 condition (Figure 6).

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 ^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	4.5
<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

Fusarium spp. had shown significantly varied spikelet bleaching severity and AUDPC on Danda'a wheat variety (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. 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 (Figure 7).

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 DAI and 28 DAI. 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 spikes [39]. On the other hand, delayed symptom development was observed by *F. ussuriarum* and *F. semitectum* after seven and 21 DAI (Figure 8).

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. 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 (**Figure 9**). 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 Canada [39].

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 Turkey [40], and root rot of corn, soybean, and wheat in Nebraska [41]. Likewise, *F. culmorum* had reported in causing higher seedling blight, while *F. graminearum* had responsible for causing severe crown rot of wheat [42-44].

Conclusion

A total of 269 single conidial purified isolates had recovered from blighted wheat spikes sampled across Jimma, Buno-Bedele, and West-Wellega zones of Oromia, southwestern Ethiopia. Based on their cultural and microscopical characteristics, all isolates had classified into nine *Fusarium* species. Among the nine specie, *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.

Acknowledgements

The authors would like to thank Ethiopian Institute of Agricultural Research and Assosa Agricultural Research Center for the financial supports. We also extend a sincere thanks to Jimma University College of Agriculture and Veterinary Medicine (JUAVM) Department of Horticulture and Plant Science for allowing us to use their plant pathology laboratory and Lath-house facilities for successful execution of this work. Also, our gratitude goes to Kulumssa Agricultural Research Center for the provision of the susceptible test crop; Danda'a wheat variety, for pathogenicity test.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Statement

This study did not involve any human or animal testing.

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