Original Research Article||

Characterization of *Colletotrichum sublineolum* Isolates and Screening of Sorghum Cultivars for their Reaction against Sorghum Anthracnose in East Hararghe

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Abstract

Anthracnose, caused by *Colletotrichum sublineolum* is an important disease of sorghum in Ethiopia. Assessing variability in a pathogen population and screening host genotypes against a particular disease is an important step in devising a sound disease management strategy. This study was aimed to characterize *C. sublineolum* isolates from three selected sorghum-growing districts (Haramaya, Kersa, and Girawa) of East Hararghe and evaluate sorghum cultivars' reaction against the disease. Infected leaf samples were collected from the three districts during 2022 cropping season. A total of 31 *Colletotrichum sublineolum* isolates were examined for their morphological and cultural traits; 11, 10, and 10 isolates were from Girawa, Kersa and Haramaya districts, respectively. Cultural and morphological characteristics of *C. sublineolum* isolates were studied by growing them on potato dextrose agar at 25 ^{o}C. Virulence of the 31 isolates was determined in three reference sets of sorghum genotypes with a detached leaf assay following a standard procedure. Twenty released sorghum cultivars and three rating reference sets of sorghum genotypes were screened in net house and laboratory conditions. The *C. sublineolum* isolates showed variation in cultural and morphological characteristics. The isolates had largely gray to light gray colony color on the upper side. The colony growth ranged from 14.50 mm to 42.33 mm. The conidial length and width ranged from 4.97 to 25.74 µm and 2.44 to 4.07 µm, respectively, with oval and falcate shapes. The virulence level varied significantly among the isolates tested. Isolates HA7A, HA3A, HA5A, HA6B, KD8C, KD8D and GM9B had higher virulence, while isolates GM2A HA1A HA4A, and KD4D had lower virulence levels on reference sorhum genotypes. The sorghum cultivars tested had significantly different responses to *C. sublineolum.* Cultivars Dekeba, Tilahun, Abshir, and Argity were identified as resistant to anthracnose disease. The finding of this study might be useful for breeding sorghum varieties for anthracnose resistance.

Key words: Anthracnose, *Colletotrichum sublineolum*, Characterize, Resistance, Sorghum bicolor, Virulence

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INTRODUCTION

Sorghum (*Sorghum bicolar*) is a drought tolerant cereal crop grown in semi-arid areas. It is used as a source of food, forage and biofuel (Girard and Awika, 2018). Sorghum serves as a source of income for the smallholder farmers producing the crop. It contains carbohydrates, iron, protein, and vitamin B3 (Palavecino et al., 2019). Sorghum is the fifth most important cereal crop in the world, following wheat, rice, maize, and barley. The global production of sorghum was 58 million tons in 2022 (FAO, 2023). In Ethiopia, four million tons of Sorghum was produced on 1.48 ha of land during 2023 (FAOSTAT, 2025). The crop accounts for 15.92% of grain production in the country (CSA, 2019). The average yield in actual, on-farm, and on-station trials was 2.4 t/ha, 3.3 t/ha, and 4.2 t/ha, respectively (Belachew et al., 2022). The production of sorghum in Ethiopia is more concentrated in the north-central, western, northwestern, and eastern mid-altitude regions (Wortmann et al., 2013). Oromia region is the major sorghum- growing region of Ethiopia contributing 42% of the country's sorghum production (USDA, 2025). Sorghum is the most important cereal crop grown in East Hararghe Zone of Oromia region.

Production and productivity of Sorghum in Ethiopia is constrained by biotic and abiotic factors. Diseases, weeds, and insects are the biotic factors limiting sorghum production in the country (Netsanet and Bewket, 2022). Anthracnose, caused by *Colletotrichum sublineolum*, is among the important production constraints of sorghum in Ethiopia (Rooney et al., 2002; Chala et al., 2010; Netsanet and Bewket, 2022). The pathogen can attack root, stalk, leaf and sheath as well as grains of sorghum (Koima et al., 2023). The disease is favored by extended periods of cloudy, warm, humid and wet weather conditions (Thakur et al., 2007). The optimum temperature range for conidia infection, germination and sporulation of *C. sublineolum* is 20-32 ^{o}C, 10-30 ^{o}C, 25-30 ^{o}C, respectively (Saloti et al., 2022). The conidia germinate on the leaf surface in the presence of water, producing germ tubes and appresoria which enables the fungus to penetrate into the host cell. (Thakur et al., 2007). The fungus survives as mycelium (on crop residue, weeds and seeds) and conidia (on seeds) (Koima et al., 2023). It may cause yield losses of 20-80% (Marley, 2004). Yield losses with a range of 26 to 35% war reported on susceptible cultivar from eastern Ethiopia (Girma et al., 2009).

Colletotrichum sublineolum is a diverse and heterogeneous fungal species (Costa et al., 2003). The first report on the presence of *C. sublineolum* races was made in 1967 and then, between 1967 and 1991, 44 different Colletotrichum spp. races or pathotypes were reported (Thakur and Mathur, 2000). Variability in *Colletotrichum sublineolum* populations in terms of morphological and cultural characteristics as well as virulence has been reported previously by different scholars (Thomas and Frederiksen, 1995; Costa et al., 2003; Souza-Paccola et al., 2003; Zanette et al., 2009; Chala et al., 2011; Tsedaley et al., 2021; and Mekonen et al., 2024). Wang et al. (2006) reported more than 40 pathotypes of *C. sublineolum* that attacked resistant sorghum genotypes over time.

Different cultural practices including, using resistant varieties, crop rotation and removal of crop residue can be used for managing sorghum anthracnose (Netsanet and Bewket, 2022). Foliar sprays of carbendazin and maneb and mancozeb and seed treatment with Apron-plus (a mixture of methalaxyl, carboxin and furathiocarpas) were effective to control the disease (Akpa et al., 1992). Among the different anthracnose management strategies available, the use of anthracnose resistant sorghum varieties is cost effective, most efficient and environmentally friendly way to control sorghum anthracnose (Abreha et al., 2021; Netsanet and Bewket, 2022). Breeding for anthracnose resistance has been a topic of research in different parts of the world including Ethiopia (Xu et al., 2020; Ridzuan et al., 2018; Mengistu et al., 2018.). Aragaw and Terefe (2024) reported promising sorghum genotypes that can be used as sources of resistance against anthracnose disease. However, the existence of variability in *C. sublineolum* population and the change in the virulence pattern of the pathogen through time makes the use of resistant varieties for anthracnose management difficult (Prom et al., 2009). Hence, continuous studies on the variability of the pathogen population along with periodic screening of local and improved sorghum genotypes to identify new sources of *C. sublineolum* resistance, are essential for

devising sustainable and effective management strategies and for resistance breeding for sorghum anthracnose disease. This study was, therefore, designed to study the variability of *Colletotrichum sublineolum* isolates collected from Eastern Hararghe and evaluate the reaction of sorghum cultivars to the pathogen.

MATERIALS AND METHODS

Description of Sample Collection Areas

The diseased plant samples collection was carried out in three districts (Haramaya, Kersa, and Girawa) which are located in East Hararghe zone, Eastern Ethiopia, during 2022 growing season. The GPS coordinates and climatic data of the districts is shown in Table 1. The map of the study districts is presented in Figure 1. .

Table 1. The GPS coordinates and climatic data of the districts from which diseased plant samples were collected

Distric	Altitude (masl)	Latitude (N)	Longitude(E)	Mean annual rainfall (mm)	Mean annual Temp.(ºC)
Haramaya	2008 - 2114	9°23'123" - 9°24'972"	41°55'99" - 42°02'215"	600- 1260	9.9- 24.2
Kersa	2088 - 2044	9°22'92" - 10°21'814"	41°55'134" -42°54'843"	410 - 1200	18-26
Girawa	1923 - 2020	9°20'621" - 9°24'88"	41°53'499" - 41°53'884"	550-1100	20-27

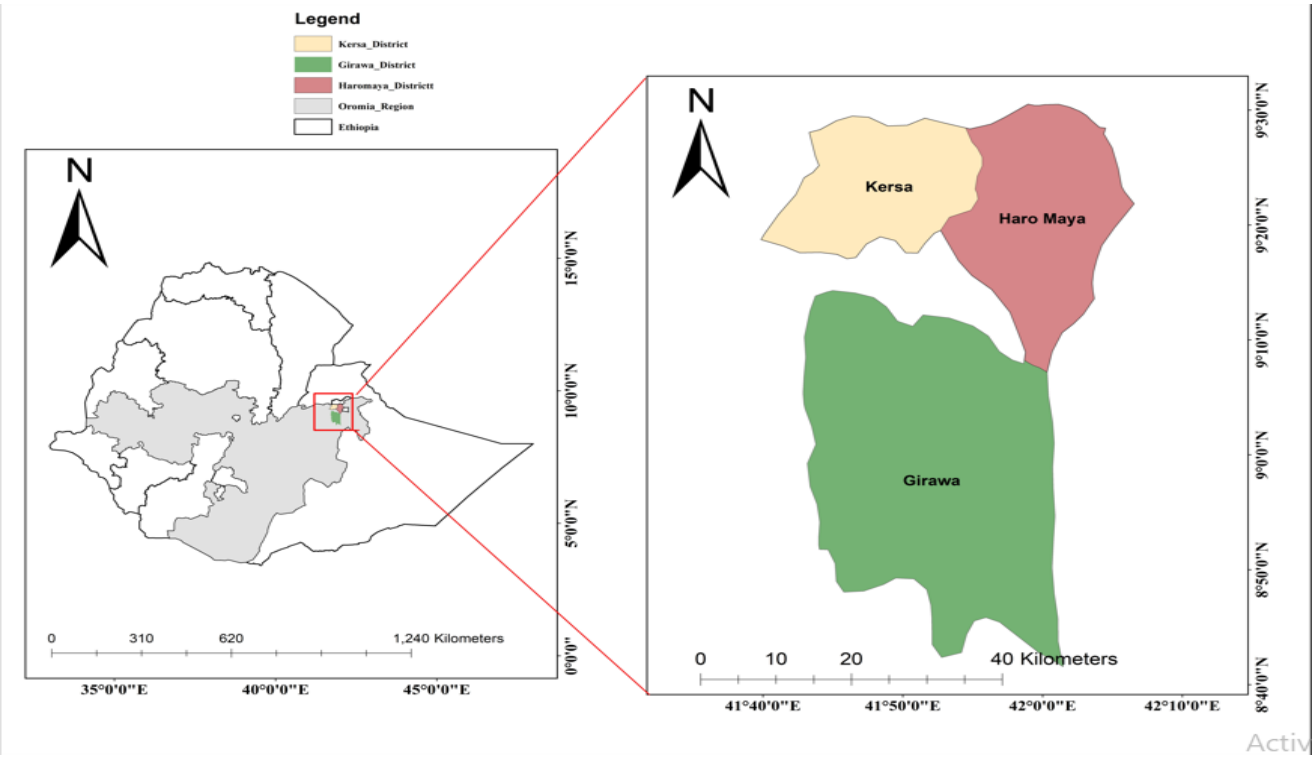

Figure 1. Map showing sample collection districts in Eastern Hararghe, Ethiopia.

Sample Collection

Sorghum fields were randomly selected at intervals ranging from 4-5km. From each district 15 to 19 sorghum samples showing typical anthracnose symptoms were collected. A total of 53 sorghum fields were visited during sample collection. Samples were kept in paper bags, and labeled with relevant information and taken to

Melkassa Agricultural Research Center Plant Pathology Laboratory for analysis.

Isolation and Identification of Sorghum Anthracnose Pathogen

Anthracnose infected Sorghum leave samples were cut into small pieces. The sample pieces were surface sterilized within 5% sodium hypochlorite solution for 3 minutes. The sterilized pieces were then rinsed three times in sterile distilled water and dried under a laminar flow hood. The dried samples were transferred into Potato Dextrose Agar (PDA) culture media and kept at 25°C for three to five days. The cultures were sub-cultured into new PDA medium to get pure cultures. Pure cultures of single spore isolates were kept at 4°C for further analysis.

Characterization of *Colletotrichum sublineolum* Isolates

For characterization study, a total of 31 *C. sublineolum* representative isolates were selected from the isolates collected from the three districts. Fungal culture of each isolate with 6mm diameter was taken from the growing margin using cork borer and placed at the center of new PDA plate. The PDA plates were then kept at 25°C in the Laboratory using CRD design with three replications for each isolate. The colony radial growth was measured five times at 48 hrs. interval. The radial growth was measured at two perpendicular angles of the reverse side of the culture plates. The other cultural and morphological characteristics studied were colony color, shape, elevation, margins, conidial size and conidial shape. Colony color was determined using the RGB (red, green, and blue) color chart (Anonymous, 2003). Width and length of conidia were measured using an Olympus-SC50 digital compound microscope. The conidial shape of the isolates was also studied by observing under a compound microscope with 10X and 40X magnifications.

Determination of the Virulence of *C. sublineolum* Isolates Using Detached Leaf Assay

The virulence study was conducted using rating reference sets (RRS) of sorghum genotypes (ETSL101173- a resistant reference set; Bonsa - a moderately resistance reference set; and BTX-623 - a susceptible reference set for *C. sublineolum* isolates). These three RRS sorghum genotypes were planted in pots filled with sterilized potting media composed of black soil, sand, and FYM mixture at a 3:2:2 ratio and kept in the net house. The description of sorghum genotypes used for the virulence study is presented in Table 2.

For preparation of inoculum for the experiment, the representative isolates were cultured on PDA medium at 25 °C for 7-10 days. The suspension of conidia for each isolate was prepared by suspending culture scraped from 7-10-day-old cultures in sterile distilled water, stirring vigorously for 90 seconds, and then filtering through two layers of cheesecloth. Before inoculation, the concentration of conidia suspension was adjusted to 1×10^6 mL^{-1} by using a hemocytometer. Healthy sorghum leaves from a 30-day-old plant were collected from the net house. The leaves were cut, surface sterilized using Clorox, and rinsed three times in sterile distilled water. The sterilized leaves were placed in Petri dishes lined with sterilized and moistened tissue papers. Individual genotypes leaves were drop-inoculated by placing 10 µl spore suspensions of each isolate and incubated on the laboratory bench until anthracnose symptoms were observed. The treatments were arranged in a factorial CRD design with three replications. The isolates virulence was determined by image analysis and a visual rating using a 1-9 scale (Thakur et al., 2007). Disease severity was recorded at 4, 7, and 10 days after inoculation (DAI). The lesion area (in square centimeters) was determined from the images using the free software ImageJ.

Table 2. Descriptions of sorghum genotypes used for the virulence study of *Colletotrichum sublineolum* isolates.

Genotypes	Resistancelevel	Source	Remark
BTX-623	Susceptible	MARC	Released
Bonsa	Medium resistance	MARC	Released
ETSL101173	Resistance	MARC	Advanced

Evaluation of Sorghum Cultivars' Reaction against *C. sublineolum*

Twenty released sorghum cultivars with three RRS genotypes were evaluated for their reaction against *C. sublineolum* under net house and laboratory conditions. The *C. sublineolum* isolates used for inoculation were chosen based on the result of pathogenicity test. Accordingly, the highly virulent isolates, HA3A, HA7A, HA5A, GM9B, and KD8C were mixed together to inoculate the cultivars tested. The isolates were mixed to have more aggressive *C. sublineolum* population and maximize the chance of getting promising resistant sorghum cultivars which may have many genes effective against several races of the *C. sublineolum*. Completely randomized design (CRD) with three replication was used for both detached leaf and intact leaf experiments.

Detached Leaf Assay

The most five highly virulent *C. sublineolum* isolates were mixed together to inoculate detached leaves of the test cultivars and RRS lines. Other inoculation procedure, data collection methods, and data type were mostly similar, as mentioned in the section on determination of virulence.

Intact Leaf Assay

The mixtures of the isolates with a 1×10^6 mL^{-1} concentration of conidia suspension was sprayed on 4-week-old sorghum plants until runoff. Inoculated plants were regularly examined for the development of *C. sublineolum* symptoms. Disease severity was evaluated by visual observation using a 1-9 scale (Table 3) (Thakur et al., 2007). The severity was assessed for five weeks at every seven-day intervals following inoculation. The Area under Disease Progress Curve (AUDPC) was calculated from the severity data using the formula below (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{Xi + Xi + 1}{2}(ti + 1 - ti)$$

Where, xi is the cumulative disease severity expressed as a proportion at the i^{th} observation, where ti is the time (days after sowing) at the i^{th} observation and n is a total number of observations.

Table 3. Disease severity scoring scale of anthracnose on sorghum

S. No	Disease severity scoring scale (1-9 scale)*	Disease reaction class
1	0 to <1% leaf area covered with hypersensitive reaction with mild yellow flecks	HR
2	1-5% leaf area covered with hypersensitive lesions without acervuli	R
3	6-10% leaf area covered with hypersensitive lesions without acervuli	R
4	11-20% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	MR
5	21-30% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	MR
6	31-40% leaf area covered with coalescing necrotic lesions with acervuli	S
7	41-50% leaf area covered with coalescing necrotic lesions with acervuli	S
8	51-75% leaf area covered with coalescing necrotic lesions with acervuli	HS
9	76-100% leaf area covered with coalescing	HS

*Disease severity score **(**Thakur et al, 2007**).**

Data Analysis

Colony growth, conidia size, disease severity, and, AUDPS data were subjected to an analysis of variance (ANOVA) using SAS software (version 9.2). Tukey's honestly significant difference test was used to separate the treatment means.

RESULTS

Cultural and Morphological Characteristics of *Colletotrichum sublineolum* Isolates

The 31 *C. sublineolum* isolates collected from Eastern Hararghe showed differences in colony color, margin, and colony elevations (Table 4 and Figure 2). Most isolates had a flat colony elevation whereas the rest had raised colony elevation. The majority of *C. sublineolum* isolates had circular colony margins, with some having irregular colony margins. The isolates formed varied colony color on the upper side and reverse side on PDA. After 10 days of growth, five types colony color on the upper side of *Colletotrichum sublineolum* isolates were observed. The colony colors formed were dirty white (6), gray (11), salmon white (5), light gray (6), and white (3). Seven different types of colony colors were recorded on reverse side as dirty white (7), dim gray (5), burlywood (6), light yellow (2), sand brown (2), light goldenrod (3), and light gray (6).

Table 4. Colony characteristics of *Colletotrichum sublineolum* isolates cultured on PDA at 25ºC for ten days.

Isolate*	Colony color		Colony Elevation[a]	Colony Margins[a]
	Upper	**Reverse**		
GM1E	Light gray	Light gray	Flat	Irregular
GM2A	Light gray	Light gray	Raised	Circular
GM3A	Salmon white	Burly wood	Raised	Circular
GM4B	Gray	Dim gray	Raised	Circular
GM5A	Light gray	Light goldenrod	Raised	Circular
GM6B	Salmon white	Light gray	Raised	Irregular
GM7A	Light gray	light gray	Raised	Circular
GM8A	Gray	Dim gray	Raised	Irregular
GM9B	Gray	Dim gray	Flat	Irregular
GM10A	Light gray	Light gray	Flat	Circular
GM11E	Gray	Dirty white	Flat	Circular
HA1A	Light gray	Burly wood	Flat	Circular
HA2B	Gray	Burly wood	Flat	Circular
HA3A	Gray	Dim gray	Raised	Circular
HA4A	Gray	Dim gray	Flat	Circular
HA5A	Dirty white	Dirty white	Flat	Irregular
HA6B	Dirty white	Dirty white	Flat	Circular
HA7A	Dirty white	Dirty white	Flat	Circular
HA8A	Dirty white	Dirty white	Flat	Circular
HA9B	Gray	Light yellow	Flat	Irregular
HA10C	Gray	Light yellow	Flat	Irregular
KD1B	Salmon white	Light goldenrod	Raised	Circular
KD2A	Salmon white	Light goldenrod	Raised	Circular
KD3E	White	Light Gray	Flat	Circular
KD4D	Salmon white	Burly wood	Raised	Circular
KD5A	White	Sandy brown	Flat	Circular
KD6A	Dirty white	Dirty white	Flat	Circular
KD7C	Gray	Burly wood	Raised	Irregular
KD8D	Dirty white	Dirty white	Flat	Irregular
KD9B	White	Sandy brown	Flat	Circular
KD10C	Gray	Burly wood	Raised	Irregular

* GM1A-GM11E isolates were obtained from Girawa, HA1A-HA10C from Haramaya, and KD1B-KD10C from Kersa

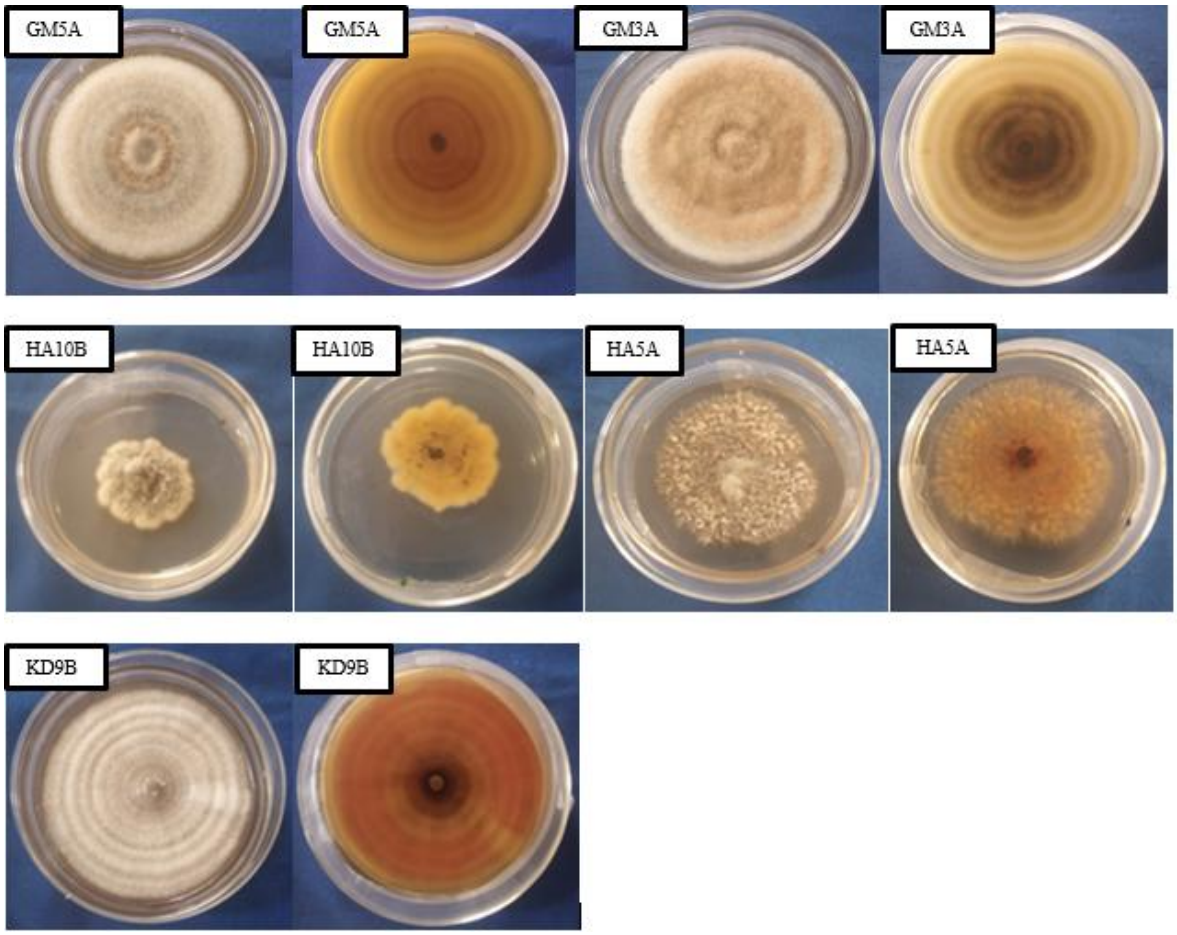

Figure 2. Cultural difference among *Colletotrichum sublineolum* isolates obtained from Eastern Hararghe after culturing on PDA at 25ºC for ten days.
Note: GM5A and-GM3A isolates from Girawa; HA10B and HA5A from Haramaya; and KD9B from Kersa.

There was significant ($p<0.0001$) variation in colony radial growth of some *C. sublineolum* isolates (Table 5). The radial growth of the isolates ranged from 14.50 mm to 42.33 mm at 10 days of incubation on PDA. Isolate KD5A had the highest mean radial colony growth (42.33 mm) at 10^{th} day although non significantly different from the radial growth of isolates GM5A, HA1A, HA1B, KD1B, KD2A,KD9B and KD10C. Whereas, HA10C isolate had the lowest radial growth of colony (14.50 mm) at 10^{th} day although not significantly different from the radial growth of isolates GM1E, GM8A, GM10A, HA4A).

Table 5. Colony radial growth of *C. sublineolum* isolates cultured on PDA at 25ºC for ten days

Isolate*	Colony radial growth (mm)[a]				
	2 Days	4 Days	6 Days	8 Days	10 Days
GM1E	8.67^{abcde}	17.00^{abcd}	21.83^{f}	28.17^{g}	33.83^{fg}
GM2A	7.83^{bcde}	16.83^{abcd}	21.00^{f}	27.67^{g}	32.83^{g}
GM3A	9.17^{abc}	19.00^{a}	29.50^{a}	37.83^{ab}	41.42^{ab}
GM4B	4.00^{hi}	5.67^{h}	9.17^{ij}	16.00^{jk}	19.00^{ijk}
GM5A	9.17^{abc}	18.67^{a}	27.83^{ab}	37.92 ^{ab}	41.50^{a}
GM6B	8.33^{abcde}	17.67^{abc}	25.83^{bcd}	33.67^{de}	37.83^{bcd}
GM7A	7.50^{de}	15.50^{cd}	21.92^{ef}	28.58^{g}	33.25^{g}
GM8A	7.83^{bcde}	17.33^{abcd}	25.58^{bcd}	32.42^{e}	36.92^{def}
GM9B	3.67^{i}	10.83^{e}	14.83^{g}	17.83^{hij}	21.17^{hij}
GM10A	7.58^{cde}	15.50^{cd}	23.33^{def}	31.50^{ef}	34.17^{efg}
GM11E	3.58^{i}	6.00^{gh}	10.00^{ij}	15.75^{jk}	18.75^{jk}
HA1A	9.50^{a}	18.42^{a}	27.50^{ab}	35.50^{bcd}	39.50^{abcd}
HA2B	9.00^{abcd}	18.00^{ab}	27.25^{abc}	34.00^{cde}	39.17^{abcd}
HA3A	5.16^{ghi}	9.83^{ef}	15.00^{g}	20.17^{h}	24.50^{h}
HA4A	5.33^{gh}	15.00^{d}	20.83^{f}	28.83^{gf}	36.33^{defg}
HA5A	5.75^{fg}	9.83^{ef}	13.67^{gh}	18.83^{hi}	24.00^{h}
HA6B	5.17^{ghi}	8.25^{fg}	11.67^{hi}	16.58^{ij}	22.50^{hi}
HA7A	4.83^{ghi}	7.08^{gh}	9.58^{ij}	13.00^{lm}	17.50^{kl}
HA8A	0.45^{ghi}	7.41^{fgh}	10.42^{ij}	13.83^{kl}	16.58^{kl}
HA9B	4.16^{ghi}	6.75^{gh}	9.17^{ij}	11.83^{lm}	15.42^{kl}
HA10C	4.16^{ghi}	6.33^{gh}	7.83^{j}	10.50^{m}	14.50^{l}
KD1B	8.83^{abcd}	18.00^{ab}	26.50^{bc}	3.40^{cde}	39.00^{abcd}
KD2A	9.42^{ab}	18.16^{ab}	26.92^{abc}	34.17^{cde}	39.00^{abcd}
KD3E	8.75^{abcde}	15.75^{bcd}	21.92^{ef}	28.17^{g}	32.83^{g}
KD4D	8.33^{abcde}	18.00^{ab}	27.33^{abc}	36.00^{abcd}	41.00^{abcd}
KD5A	7.83^{bcde}	17.83^{abc}	27.67^{ab}	38.33^{a}	42.33^{a}
KD6A	5.00^{ghi}	11.50^{e}	15.67^{g}	18.92^{hi}	23.83^{h}
KD7C	8.83^{abcd}	17.67^{abc}	26.33^{bc}	33.67^{de}	37.50^{cde}
KD8D	5.00^{ghi}	12.00^{e}	16.18^{g}	18.92^{hi}	23.83^{h}
KD9B	7.19^{ef}	17.58^{abc}	27.50^{ab}	36.58^{abc}	41.42^{ab}
KD10C	7.67^{cde}	17.16^{abcd}	24.67^{cde}	33.75^{de}	39.08^{abcd}
CV (%)	7.35	5.45	4.32	3.15	3.62
LSD	1.62	2.44	2.80	2.69	3.62

*GM1E -GM11E isolates from Girawa; HA1A -HA10C isolates from Haramaya; and KD1B -KD10C isolates from kersa. CV (%) is the coefficient of variation. LSD= Least Significant Difference. Mean values with the same letter within a column do not differ significantly at 5% level of significance.

[a] Colony radial growth was measured at 2 days interval, .

There was a highly significant ($p<0.0001$) variation in conidia size between isolates of *C. sublineolum* (Table 6). The mean conidia length and width ranged from 4.97 to 25.74µm and 2.44 to 4.07 µm, respectively. Isolate HA3A had the highest mean conidial length (25.74 µm) and width (4.07 µm), while isolate KD7C had the lowest mean conidial length (4.97 µm) and width (2.44 µm) although not significantly different from the conidial length and width of

some of isolates. The *C. sublineolum* isolates produced two types of conidia; namely, falcate and oval shapes (Table 6 and Figure 3). The falcate conidia shape is larger than the oval type. However, there was no significant variation in conidia length among the falcate conidia shapes of the different isolates except for GM9B. The oval conidia shape was of small conidia size and no significant variation among the isolates that produced oval conidia shape. The majority of *C. sublineolum* isolates (20) that infected sorghum had oval conidia shape while the remaining 11 isolates had a falcate conidia shape.

Table 6. The conidial characteristics of *Colletotrichum sublineolum* isolates from eastern Hararghe after ten days of incubation.

Isolate*	Conidia shape	Conidia size (µm)[a]	
		Length	Width
GM1E	Oval	5.03^{c}	2.44^{d}
GM2A	Oval	5.39^{c}	2.76^{d}
GM3A	Oval	5.67^{c}	2.56^{d}
GM4B	Falcate	25.53^{a}	3.76^{abc}
GM5A	Oval	5.54^{c}	2.58^{d}
GM6B	Oval	5.40^{c}	2.64^{d}
GM7A	Oval	5.76^{c}	2.68^{d}
GM8A	Oval	5.28^{c}	2.50^{d}
GM9B	Falcate	19.74^{b}	3.83^{abc}
GM10A	Oval	5.13^{c}	2.51^{d}
GM11E	Falcate	25.65^{a}	3.76^{abc}
HA1A	Oval	5.50^{c}	2. 46^{d}
HA2B	Oval	5.62^{c}	2.71^{d}
HA3A	Falcate	25.74^{a}	4.07^{a}
HA4A	Oval	5.14^{c}	2.60^{d}
HA5A	Falcate	25.24^{a}	3.91^{ab}
HA6B	Falcate	24.67^{a}	3.54^{bc}
HA7A	Falcate	25.39^{a}	3.70^{abc}
HA8A	Falcate	23.56^{a}	3.38^{c}
HA9B	Falcate	24.42^{a}	3.78^{abc}
HA10C	Falcate	23.73^{a}	3.86^{abc}
KD1B	Oval	5.40^{c}	2.55^{d}
KD2A	Oval	5.31^{c}	2.49^{d}
KD3E	Oval	5.59^{c}	2.50^{d}
KD4D	Oval	5.67^{c}	2.72^{d}
KD5A	Oval	5.87^{c}	2.63^{d}
KD6A	Falcate	24.72^{a}	3.67^{abc}
KD7C	Oval	4.97^{c}	2.44^{d}
KD8D	Falcate	23.95^{a}	3.66^{abc}
KD9B	Oval	5.31^{c}	24.47^{d}
KD10C	Oval	7.20^{c}	2.78^{d}
CV (%)		**6.28**	**5.10**
LSD		**2.59**	**0.49**

*GM1E-GM11E isolates from Girawa; HA1A-HA10C isolates from Haramaya; and KD1B-KD10C isolates from Kersa. LSD= Least Significant Difference. CV (%) is the coefficient of variation.

[a]Means followed by the same letter within a column is not significantly different from each other.

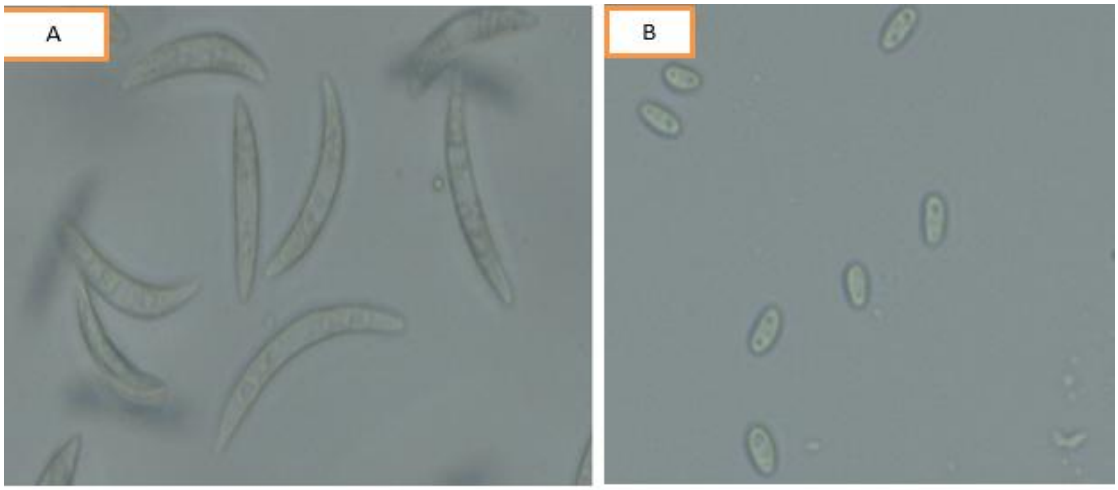

Figure 3. Morphological variation in the conidia of *Colletotrichum sublineolum* isolates after ten days of incubation. A=falcate conidia (HA3A); B=oval conidia (HA7C).

Determination of the Virulence of *Colletotrichum sublineolum* Isolates on Detached Sorghum Leaves

The result of this study demonstrated that the isolates from various locations of Eastern Hararghe had varying virulence levels on the three sorghum rating reference sets tested on detached leaves (Table 7, Figure 4). The mean area of lesion on the sorghum genotypes due to the disease varied from 0 cm^2 to 6.09 cm^2 after 10 days of inoculation. HA7A isolate had the largest mean lesion area (6.09 cm^2), while isolates GM2A and KD4D had the smallest mean lesion area (0.00 cm^2) in all tested sorghum genotypes. The disease severity score was between 1.0 and 7.33 after 10 days of inoculation. Isolate HA7A inoculated to genotype BTX-623 resulted in the highest mean severity score (7.33), while GM2A and KD4D isolates had a low mean severity score (1.00) in all sorghum genotypes tested. The genotype ETSL101173 had resistant to moderately resistant reaction to all the isolates. In this study, high virulence was detected in the interaction of all three genotypes with the isolates HA7A, HA3A, HA5A, and KD8C. Low virulence was the results of all genotypes interacting with GM2A, HA1A, HA10C and KD4D isolates. The genotypes BTX-623 and Bonsa showed susceptible reaction to isolates, GM9B, HA3A, HA5A, HA6B, HA7A, HA8B, KD7C and KD8D. All the three genotypes tested showed resistant reaction to isolates, GM2A, HA1A, HA4A, and KD4D. Overall, most of isolates were highly virulent on BTX-623 genotype compared to the other two genotypes.

Table 7. Interaction of *Colletotrichum sublineolum* isolates with Sorghum Genotypes in the development of Sorghum Anthracnose on detached leaves

Isolates[a]	BTX-623			BONSA			ETSL101173		
	Lesion Area	Severity Scale	Disease reaction	Lesion Area	Severity Scale	Disease reaction	Lesion Area	Severity Score	Reaction type
GM1E	3.37	4.67	MR	0.12	1.33	HR	0.00	1.00	HR
GM2A	0.00	1.00	HR	0.00	1.00	HR	0.00	1.00	HR
GM3A	1.00	3.00	R	2.72	4.67	MR	1.53	4.00	MR
GM4B	4.26	6.00	S	2.11	4.33	MR	0.98	2.33	R
GM5A	4.22	6.33	S	3.21	4.67	MR	2.64	4.00	MR
GM6B	4.25	6.33	S	3.53	5.67	MR	3.41	5.67	MR
GM7A	0.43	2.33	R	3.93	5.67	MR	0.00	1.00	HR
GM8A	2.94	4.33	MR	2.12	4.33	MR	0.00	1.00	HR
GM9B	5.47	6.67	S	4.80	6.33	S	3.67	5.33	MR
GM10A	2.36	4.33	MR	1.59	4.00	MR	1.60	4.00	MR
GM11E	4.86	6.33	S	2.30	4.33	MR	1.40	3.33	R
HA1A	1.27	3.67	R	0.98	3.00	R	0.78	2.67	R
HA2B	2.69	4.33	MR	0.48	2.00	R	0.00	1.00	HR
HA3A	5.62	7.00	S	4.69	6.33	S	3.77	5.67	MR
HA4A	0.50	2.33	R	1.15	3.00	R	0.00	1.00	HR
HA5A	5.54	7.00	S	4.90	6.33	S	3.89	5.33	MR
HA6B	4.38	6.33	S	4.55	6.00	S	1.27	3.33	R
HA7A	6.09	7.33	S	5.47	6.67	S	4.02	5.33	MR
HA8B	4.46	6.33	S	4.41	6.00	S	1.35	3.33	R
HA9A	4.04	5.67	MR	1.72	4.00	MR	1.60	4.00	MR
HA10C	1.40	3.67	R	1.57	4.00	MR	2.22	4.33	MR
KD1B	3.15	5.33	MR	1.38	3.67	R	0.00	1.00	HR
KD2A	3.07	4.33	MR	1.83	4.00	MR	3.16	5.33	MR
KD3E	2.74	4.67	MR	0.00	1.00	HR	1.31	3.67	R
KD4D	0.00	1.00	HR	0.00	1.00	HR	0.00	1.00	HR
KD5A	4.37	6.00	S	3.99	5.67	MR	1.38	3.67	R
KD6A	4.70	6.33	S	1.60	4.00	MR	1.31	3.67	R
KD7C	4.36	6.00	S	4.25	6.00	S	1.22	3.67	R
KD8D	5.58	7.00	S	5.07	6.67	S	3.65	5.67	MR
KD9B	2.54	4.33	MR	2.70	4.67	MR	1.13	3.33	R
KD10C	3.21	4.33	MR	3.31	4.00	MR	0.00	1.00	HR

CV of severity score =13.59; CV of lesion area = 14.88

[a]GM1E-GM11E isolates from Girawa; HA1A-HA10B isolates from Haramaya; and KD1B-KD10C isolates from Kersa.

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.

CV (%) = coefficient of variation;

Mean values with the same letter within a column are not significantly different from each other.

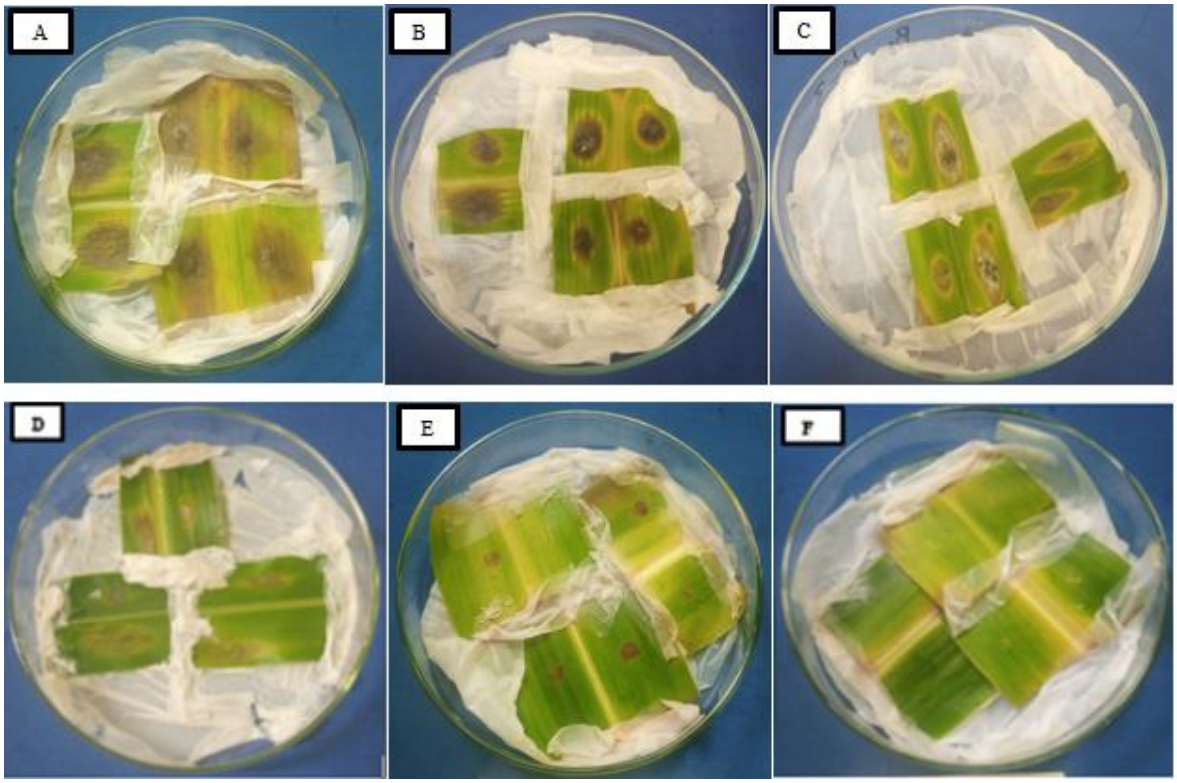

Figure 4. Interaction of *Colletotrichum sublineolum* isolates with Sorghum rating reference sets.
A= HA7A isolate with BTX-623; B = HA7A isolate with Bonsa; C = HA3A isolate with BTX623; D= GM10A isolate with BTX-623; E = HA1A isolate with ETSL101173; and F = KD4D with ETLSL101173.

Responses of Sorghum Cultivars to *Colletotrichum sublineolum* Isolates

Detached Leaf Assay

The result of this study showed significant ($p<0.0001$) variation among sorghum cultivars reaction against *Colletotrichum sublineolum* isolates (Table 8). The reaction of sorghum cultivars against *Colletotrichum sublineolum* isolates in the detached leaf test is presented in Figure 5**.** The lesion area ranged from zero to 7.21 cm^2. The cultivar Abshir had the lowest mean lesion area, while Adelle had the highest mean lesion area. On the other hand, the disease severity score ranged from one to 8.67. The mean disease severity score of the Abshir cultivar was the lowest (1), while that of Adelle was the highest (8.67). Based on the finding of the detached leaf assay, Sorghum cultivars, Adelle, Dibaba, Jiru, Chiro, Dagim, Seredo, Gambella-1107, BTX-623 and Bonsa cutivars were susceptible to the disease. Among these, Adelle and Dibaba cultivars showed highly susceptible reactions and had disease severity scores of 8.67 and 8.00, respectively. The resistant checks, ETSL101173 and nine other sorghum cultivars displayed moderate resistant reaction against *Colletotrichum sublineolum.* Abshir, Tilahun, Argity, and Dekeba cultivars with disease severity scores of 1.00, 2.33, 3.33, and 3.67, respectively showed reactions ranging from resistant to highly resistant against the pathogen.

Table 8. Sorghum cultivars' reaction to *Colletotrichum sublineolum* in the detached leaf test.

Cultivar	Lension area (cm^2)*	Severity score*	Disease reaction type
Abshir	0.00^{j}	1.00^{j}	HR
Adelle	7.21^{a}	8.67^{a}	HS
Argity	1.37^{ij}	3.33^{hi}	R
Baji	3.09^{efgh}	4.33^{fghi}	MR
Berhan	4.09^{def}	5.67^{cdefg}	MR
Birmash	2.33^{ghi}	4.33^{fghi}	MR
Bonsa	5.61^{bcd}	6.66^{abcde}	S
BTX-623	6.70^{ab}	7.67^{abc}	S
Chiro	5.46^{bcd}	6.67^{abcde}	S
Dagim	4.35^{cde}	6.00^{bcdef}	S
Dibaba	6.89^{ab}	8.00^{ab}	HS
Dekeba	1.32^{ij}	3.67^{ghi}	R
ETSL101173	4.08^{def}	5.67^{cdefg}	MR
Gambella-1107	5.86^{abc}	7.33^{abcd}	S
Gobiye	2.50^{ghi}	4.67^{efgh}	MR
Jiru	5.71^{abc}	7.00^{abcd}	S
Macia	4.11^{def}	5.67^{cdefg}	MR
Meko	3.31^{efg}	5.33^{defgh}	MR
Melkam	2.73^{efgh}	4.67^{efgh}	MR
Seredo	6.70^{ab}	7.33^{abcd}	S
Teshale	2.94^{efgh}	4.00^{fghi}	MR
Tilahun	0.62^{j}	2.33^{ij}	R
76Ti#23	1.56^{hij}	4.00^{fghi}	MR
CV (%)	13.04	12.27	
LSD	1.57	2.07	

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.
CV (%) = coefficient of variation; LSD=Least Significant Difference
Mean values with the same letter within a column are not significantly different from each other.

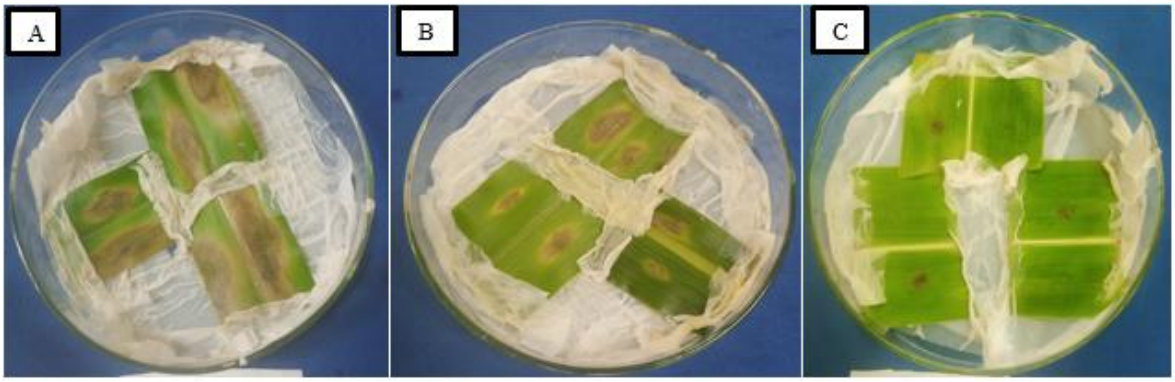

Figure 5. Reaction of sorghum cultivars against *Colletotrichum sublineolum* in the laboratory. A = highly susceptible (Adelle). B=moderately resistant (Melkam); C=resistant (Tilahun)

Intact Leaf Assay

Host Response to Sorghum Anthracnose Disease under Net House Conditions

Based on the finding of this study, significant ($p<0.0001$) differences were observed among sorghum cultivars response to *Colletotrichum sublineolum* isolates under net house conditions (Table 9). The sorghum cultivars were divided into different disease reaction types based on their disease severity score which ranged from 1.00 to 8.33. The reaction of sorghum cultivars against a mixture of *Colletotrichum sublineolum* isolates under net house conditions is presented in Figure 6. Anthracnose symptoms were observed in all cultivars except Tilahun and Abshir that were highly resistant to the disease. Three cultivars, namely Seredo, Dibaba, and Adelle, with disease severity scores of 8.33, 8.11, and 8.00, respectively were classified as highly susceptible. Around seven cultivars, including the universally susceptible check (BTX-623), were found to be susceptible to the disease. Eight cultivars, including the resistance check (ETSL101173) and the moderate resistant check (Bonsa), had moderately resistant reactions. On the other hand, Gobiye, Dekeba, and Argity cultivars with disease severity scores of 3.89, 3.89, and 2.89, respectively were resistant to anthracnose disease.

Table 9. Average disease severity scores and types of disease reactions on some sorghum cultivars against *Colletotrichum sublineolum* under net house conditions

Cultivar	Severity (1-9)	Reaction type*
Abshir	1.00^{j}	HR
Adelle	8.00^{abc}	HS
Argity	2.89^{i}	R
Baji	4.22^{hi}	MR
Berhan	6.33^{bcdef}	S
Birmash	5.11^{efgh}	MR
Bonsa	5.56^{defgh}	MR
BTX-623	6.56^{abcde}	S
Chiro	6.78^{abcd}	S
Dagim	6.11^{defg}	S
Dibaba	8.11^{ab}	HS
Dekeba	3.89^{hi}	R
ETSL101173	4.67^{fghi}	MR
Gambella-1107	6.22^{cdef}	S
Gobiye	3.89^{hi}	R
Jiru	7.67^{abcde}	S
Macia	4.56^{fghi}	MR
Meko	6.11^{defg}	S
Melkam	4.33^{ghi}	MR
Seredo	8.33^{a}	HS
Teshale	5.22^{efgh}	MR
Tilahun	1.00^{j}	HR
76Ti#23	4.11^{hi}	MR
CV (%)	11.26	
LSD	1.86	

*Reaction type: HR=highly resistant, R=resistant; MR=moderately resistant; S=susceptible; and HS=highly susceptible. Classification of cultivars to reaction type based on severity score: 1 = classified as highly resistant (R); 2&-3 = resistant; 4&5 =classified as moderately resistant; 6&7 = classified as susceptible; and 8&9 = classified as highly susceptible.

CV (%) = coefficient of variation; LSD=Least Significant Difference

Mean values with the same letter within a column are not significantly different from each other.

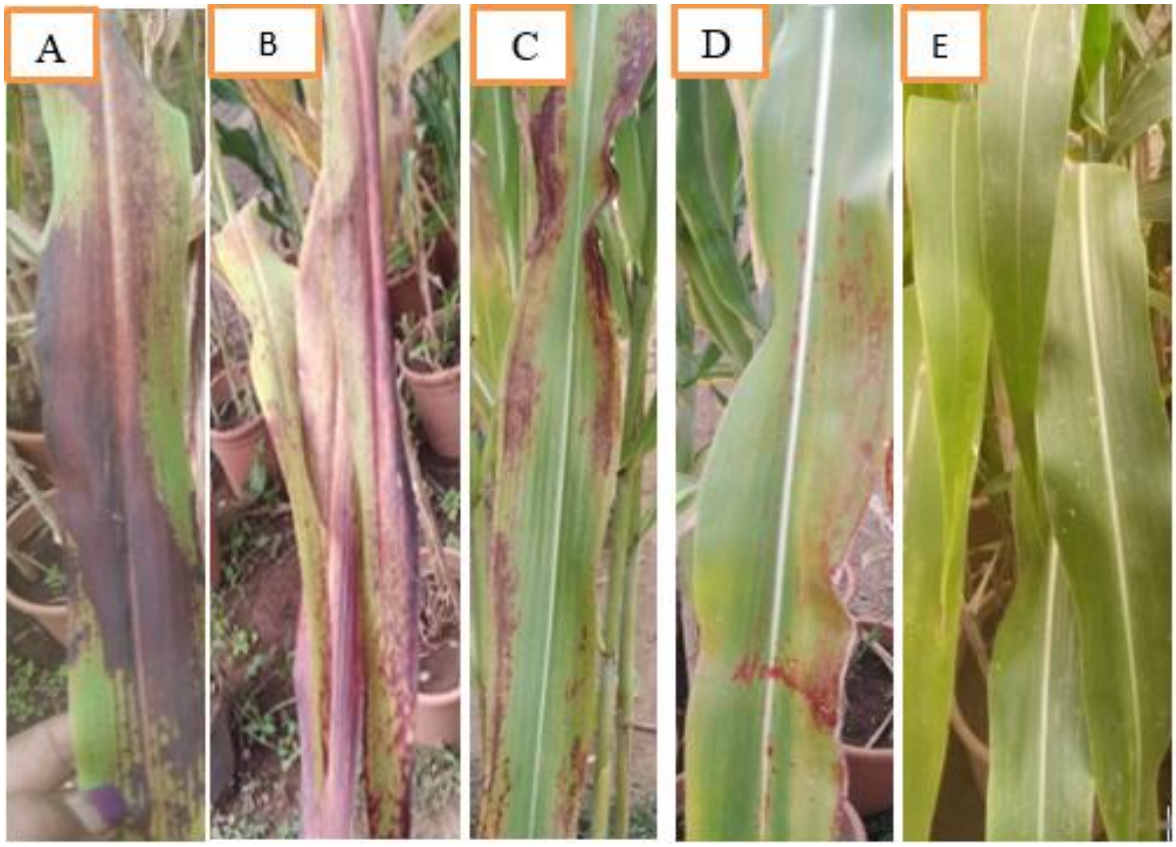

Figure 6. Reaction of sorghum cultivars against a mixture of *Colletotrichum sublineolum* isolates under net house conditions. A and B = highly susceptible; C=susceptible; D= moderately resistant; and E=resistant

Percent severity index and area under disease progress curve (AUDPC) of Anthracnose

The percent severity index and area under disease progress curve of anthracnose disease on sorghum cultivars grown under net house conditions is presented in Table 10. There was significant variations ($p<0.0001$) in percent severity index within the sorghum cultivars tested. The final percent severity index (FPSI) value for anthracnose disease ranged from 11.11% to 92.59%. The cultivar Seredo had the highest FPSI value (92.59%) although not significantly different from FPSI values of Adelle, BTX-623, Chiro, Dibaba, Jiru and Seredo. On the other hand, cultivars Tilahun and Abshir had significantly ($P<0.0001$) lower FPSI value (11.11%) compared to FPSI values of other genotypes.

The area under the disease progress curve showed a highly significant ($p<0.0001$) difference between the tested sorghum genotypes. The AUDPC values of the cultivars tested ranged from 311.11%-days to 1597.77 %-days. The highest AUDPC value (1598.77 %-days) was computed for cultivar Dibaba, though not significantly different from AUDPC values of Adelle, BTX623, Chiro, Jiru, and Seredo cultivars. On the other hand, significantly lower AUDPC value (311.11%-day) was observed on cultivars Tilahun and Abshir compared to the values of the other sorghum cultivars (Table 8).

Table 10. The mean percent severity index (PSI) and area under progress curve (AUDPC) of *Colletotrichum sublineolum* on sorghum cultivars under net house conditions.

Cultivar	IPSI (%)*	FPSI (%)*	AUDPC (%-days)*
Abshir	11.11^{c}	11.11^{j}	311.11^{i}
Adelle	24.69^{a}	88.89^{abc}	1564.20^{a}
Argity	11.11c	32.10^{i}	566.05^{hi}
Baji	16.05^{bc}	46.91^{hi}	980.86^{defg}
Berhan	18.52^{abc}	70.37^{bcdef}	1175.61^{bcde}
Birmash	11.11^{c}	56.79^{efgh}	946.30^{defg}
Bonsa	20.99^{ab}	61.73^{efgh}	1162.75^{bcdef}
BTX-623	18.52 ^{abc}	72.84^{abcde}	1270.37^{abcd}
Chiro	24.69^{a}	75.31^{abcde}	1421.60^{ab}
Dagim	13.58^{bc}	67.90^{defg}	1158.02^{bcdef}
Dibaba	20.99^{ab}	90.12^{ab}	1598.77^{a}
Dekeba	11.11^{c}	43.21^{hi}	795.06^{gh}
ETSL101173	14.81^{bc}	51.85^{fghi}	959.26^{defg}
Gambella-1107	13.58^{bc}	69.14^{cdef}	1153.70^{bcdef}
Gobiye	11.11^{c}	43.21^{hi}	786.42^{gh}
Jiru	17.28^{abc}	85.19^{abcd}	1326.54^{abc}
Macia	14.81^{bc}	50.62^{fghi}	989.51^{cdefg}
Meko	11.11^{c}	67.90^{defg}	1088.89^{bcdefg}
Melkam	11.11^{c}	48.15^{ghi}	829.63^{fgh}
Seredo	18.52^{abc}	92.59^{a}	1529.63^{a}
Teshale	13.58^{bc}	58.03^{efgh}	993.83^{cdefg}
Tilahun	11.11^{c}	11.11^{j}	311.11^{i}
76Ti#23	17.29^{abc}	45.68^{hi}	885.80^{efgh}
CV (%)	**15.79**	**11.26**	**10.45**
LSD	**7.70**	**20.62**	**339.92**

AUDPC= Area Under Disease Progress Curve; IPSI=initial percent severity index, FPSI=Final percent severity index; CV (%) =coefficient of variation. LSD= Least Significant Difference.
*The mean values in a column that have the same letters are not significantly different from one another.

DISCUSSION

Sorghum is an important cereal crop supporting the livelihood of millions of smallholder farmers in Ethiopia. However, its productivity is constrained by biotic and abiotic factors. Sorghum anthracnose (*Colletotrichum sublineolum*) is one of the most serious diseases affecting sorghum production in Ethiopia in general and East Hararghe in particular. Due to the variable nature of the pathogen population, it is critical to periodically study variability in the pathogen population and screen cultivars against the pathogen to devise a sound management strategy for the disease. The result of this study revealed that there was variation in cultural and morphological characteristics of *Colletotrichum sublineolum* isolates collected from East Hararghe. The isolates showed variation in colony colors, margins, and elevations.

Most of the isolates had gray to light gray colony color on the upper surface. In general, seven different reverse side colony colors of *C. sublineolum* isolates were observed in this study. Similarly, Aragaw et al. (2023) reported differences in cultural and morphological characteristics of *C. sublineolum* isolates obtained from Eastern Ethiopia. Tsedaley et al. (2016) also reported variability in the colony characteristics of *C. sublineolum* isolates collected from south western and western Ethiopia. The colonies had whitish gray and yellow to purple gray color on the upper sideds of the petri dishes and goldenrod, brownish, purple grayish, and whitish colors on the reverse side of the petri dishes. In another study by Were and Ochuodho (2012), The mycelial color of all *C. sublineolum* isolates was gray, but with various color intensities on upper and rever side of culture plate.

The mean colony radial growth of *Colletotrichum sublineolum* isolates obtained in this study varied significantly and ranged from 14.50 mm to 42.33 mm. Significant differences in radial growth of *C. sublineolum* isolates was also reported by Chala et al. (2011). Zanette et al. (2009) reported *C. sublineolum* isolates with varied radial growth. The variations in radial colony growth of the *C. sublineolum* isolates could be due to the pathogen's genetic diversity.

The conidial size of *C. sublineolum* isolates varied significantly and the mean conidial length and width ranged from 4.97 to 25.74 µm and 2.44 to 4.07 µm, respectively. Significant variations among the *C. sublineolum* isolates in terms of conidia width and length was reported by Mekonen et al. (2024). Two different types of conidial shape, falcate and oval were produced by *C. sublineolum* isolates in the present study with the majority of isolates having oval-shaped conidia. Falcate and oval type of conidia of *C. sublineolum* was also reported by Souza-Paccola et al. (2003) and Thomas and Frederiksen (1995). Chala et al. (2011) also reported morphological differences in the conidial shape of *C. sublineolum* isolates. Zanette et al. (2009) reported variation in conidial morphology of *C. sublineolum* isolates and explained that the variation might be due to pathogen's attempt to overcome panicle resistance to infection. The variations in cultural and morphological characteristics of *C. sublineolum* isolates in the present study might be related to the pathogen's diversity, environment and sorghum genotypes.

The result of the virulence test showed variation in virulence of *C. sublineolum* isolates tested on reference sorghum sets. This implies the presence of different pathotypes of *C. sublineolum* infecting sorghum. Based on the finding, isolates HA7A, HA3A, HA5A, KD8C, and GM9B had higher levels of virulence. Overall, the isolates collected from sorghum growing fields in Haramaya district had higher virulence response than those isolates from the other locations in East Hararghe. High anthracnose severity from Haramaya district was also reported previously (Aragaw et al., 2019). Differences in virulence and aggressiveness in *C. sublineolum* isolates was also previously reported from Ethiopia (Mekonen et al., 2024; Tsedaley et al., 2021). The variations in virulence among *C. sublineolum* isolates could be due to the genetic variation in the pathogen population, genotypes used for the test and the difference in environmental variables in the areas from which the isolates were collected.

Although different management options including resistant varieties, crop rotation, removal of crop residue and chemicals are available for managing sorghum anthracnose, the use of resistant varieties is cost effective, and environmental friendly strategy for the management of sorghum anthracnose (Abreha et al., 2021). Hence, screening of sorghum germplasm against anthracnose disease is crucial to look for source of resistance for the disease. The result of the screening test in this study revealed that there was significant variation among sorghum cultivars' reaction to *C. sublineolum.* Variation in sorghum genotypes reaction to anthracnose disease was also reported previously (Chala and Tronsmo, 2012; Prom et al., 2012); Cuevas et al., 2014 and Cuevas et al., 2016; Koima et al., 2023). Disease severity and the area under disease progress curve varied amongst the tested sorghum cultivars. The majority of the cultivars screened were either susceptible or moderately resistant to the disease with relatively few cultivars showing resistant responses. Different responses to the anthracnose disease were observed under both net house and laboratory conditions in five cultivars (Berhan, Gobiye, Meko, Seredo, and Tilahun) and the moderately resistant check Bonsa. However, Dekeba, Tilahun, Abshir, and Argity showed a highly resistant response to anthracnose disease under both conditions. Erpelding (2010) documented very susceptible, susceptible, and resistant responses of sorghum accessions to *C. sublineolum* isolates. Prom et al. (2016) found that all sorghum lines except one were susceptible when inoculated with a mixture of *C. sublineolum* isolates in detached leaf assay. The variation in responses of the sorghum genotypes tested for anthracnose disease in the present study might indicate the presence of genetic variation for host resistance.

CONCLUSIONS

There was variation in the morphological and cultural characteristics as well as the virulence level of the *C. sublinelum* isolates collected from East Hararghe. Sorghum genotypes screened in this study varied in their reaction to anthracnose disease. Four sorghum cultivars, namely Dekeba, Tilahun, Abshir, and Argity were resistant to *C. sublineolum* under net house and laboratory conditions. The finding of this study would serve as baseline information for the national breeding program for breeding for sorghum anthracnose resistance. Conducting variability studies of pathogen populations with molecular tools would be important to overcome limitations associated with morphological pathogen variability studies. To come

up with a more concrete conclusions about the reactions of the genotypes tested for anthracnose disease, repeating the experiment under field condition would be of paramount importance.

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