Cultural, Morphological and Pathological Characterization of Alternaria Porri Isolates on Onion (Allium cepa L.) in Central and Eastern Ethiopia

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Abstract

Alternaria porri (Ellis) Cif., the causative agent of onion purple blotch, causes severe damage to onion production in Ethiopia. However, there has been no empirical data on characterization of the isolates from Ethiopia. Eighteen *A. porri* isolates were collected from different onion-growing districts of Central and Eastern Ethiopia in 2016 cropping season to determine variation among the isolates using cultural, morphological and pathogenic traits. A 6-mm mycelia-disc was taken from a 7-day-old culture grown on potato dextrose agar (PDA) of each isolate and placed in center of a 90-mm petri-dish containing PDA medium. Three cultures were kept for each isolate, in a completely randomized design (CRD) and incubated at 25 ^{o}C for 7-15-days. A conidial suspension ($5x10^4$/ml) was made from a 10-day-old culture of each isolate and inoculated by spraying on 60-day-old seedlings of onion cv. Adama Red grown in plastic-pots (20-cm-diameter). The inoculated plants (two/pot) were arranged in a CRD in three replications. The isolates significantly ($p \leq 0.001$) varied for cultural, morphological and pathological traits. Colony growth rate of the isolates ranged between 0.28 and 0.65 mm/day. Color of the colonies ranged from whitish-gray to dark-gray and had velvety or cottony texture with circular to irregular margins. The conidial dimensions ranged from 19.86-38.73 x 8.86-12.83 µm, with 2-5 transverse and 1-2 longitudinal septa. The area under disease progress curve and disease progress rate levels ranged from 267.20-1068.23%-days and 0.08-0.19 unit/day, respectively. The study has identified existence of considerable variation among *A. porri* isolates in Central and Eastern Ethiopia.

Keywords: *Alternaria porri*; onion; pathogen characterization

1. Introduction

Onion (*Allium cepa* L.), is one of the most widely cultivated vegetable crops in the world. It ranked third following tomato and cabbage with respect to a world total production of 85.8 million tons and cultivated area of 4.4 million ha (FAO, 2013). In Ethiopia, it is largely grown by small-scale farmers in diverse agro-ecological regions both under rain-fed and irrigated conditions and occupies an area of about 0.03 million ha with total production of 3.27 million tons in 2016/2017 main cropping season (CSA, 2017). The majority of onion production is found in the Rift Valley areas, mainly along the Awash River and around the Rift Valley Lakes (Olani and Fikre, 2010). The onion bulbs and lower sections of the stems are the most popular as a flavoring condiment in daily dish of every Ethiopians and believed to be more intensively consumed than any other vegetable crop (Negasi *et al*., 2013). In addition to this, it serves as a good source of income for those people who involve in production, processing and marketing value chains and helps them to improve their livelihood, and also regarded as a highly export oriented crop and earn valuable foreign exchange for the country (Joosten *et al*., 2011).

In spite of its multi-dimensional functions, the national average yield of onion under subsistence production is about 9.75 t ha^{-1} (CSA, 2017), which is much lower than the world average yield of 19.50 t ha^{-1} (FAO, 2013). The low yield as identified by diagnostic studies is mainly attributed to fungal diseases like purple blotch, downy mildew and basal rot (Wondirad *et al.*, 2009; Haile *et al*., 2016). Among the fungal diseases, purple blotch (PB) caused by *Alternaria porri* (Ellis) Cif., is the most prevalent and severe disease in nearly all major onion-producing areas of the world. The disease commonly attacks leaves and flower stalks to become blight and die prematurely, limits the photosynthetic area available, and thereby causes considerable yield reduction mainly during extended periods of warm and wet weather (Yadav *et al.*, 2013). Yield losses of onion bulb and seed crops in India due to PB were estimated to be about 59% (Gupta and Pathak, 1988) and 97% (Lokra, 1999), respectively. In Ethiopia, yield loss due to this disease was estimated to reach 50% of the total production (Lemma and Shimeles, 2003).

Management options for PB of onion include use of pathogen-free seeds or seedlings, removal or burning of infected plant debris and alternate *Allium* weed hosts, rotations with non-host crops (Schwartz, 2014), cultivation of resistant varieties (Abubakar and Ado, 2013) and fungicide treatments (Wanggikar *et al*., 2014). Since onion production intensified over the past few

decades, growers become more dependent on spraying of fungicides (e.g., mancozeb 50% WP at 3 kg ha^{-1}) as a relatively reliable method of crop protection. About 5-7 sprays are applied per growing season depending on weather conditions and the intensity of the disease (Lemma and Shimeles, 2003). However, it is known that repeated use of fungicides alone increases cost of production, leads to accumulation of toxic residues in produce and environment, resulting in risks to human and animal health, and development of new pathogen races or pathotypes (Burkett-Cadena *et al*., 2008; Behera *et al.,* 2013) through selection pressure, which could result in even the worst economic and environmental problems.

Understanding of pathogen variability in a population on the basis of cultural, morphological and pathological traits is helpful for designing strategies for more effective, economical and sustainable disease management by facilitating selection for desirable traits (Chowdappa *et al*., 2012; Sofi *et al*., 2013). In Bangladesh, Mohsin *et al*. (2016) indicated the occurrence of different physiological races among *A. porri* isolates of onion PB based on cultural, morphological and pathological traits. In spite of the severe damage caused by PB to onion production and productivity in Ethiopia, there has been lack of detailed experimental data on variability of the pathogen isolates. Therefore, the objective of this study was to determine the existence of cultural, morphological and pathological variations among *A. porri* isolates of onion PB collected from various locations in Central and Eastern Ethiopia.

2. Materials and methods

2.1. Description of the study areas

Onion leaf samples showing typical symptoms of *A. porri* (sunken purple lesions that are often elliptical with a yellow to pale-brown margin) were collected from seven major onion-growing districts (Adama, Adami-Tulu-Jido-Kombolcha, Bora, Dugda, Haramaya, Kersa and Lume) of Central and Eastern Ethiopia in 2016 main cropping season. The districts are located in the East Hararghe and East Shewa administrative zones of Oromia Region, between 07°58′ and 09°26’ N latitudes, 038°43’ and 041°57′ E longitudes, with an altitudes ranging from 1543-1997 meters above sea level. The areas have an arid to semi-arid climate with mean annual temperatures and rain-falls in the range of 17-25 ^{o}C and 600-1700 mm, respectively (District Agriculture Development Office). Brief description of the geographic features and climate characteristics of the study areas are presented in Table 1.

Table 1: Geographical features and climatic characteristics of the study areas in East Shewa and East Hararghe Zones, Ethiopia

Zone	District	Altitude (masl)	Latitude (N)	Longitude (E)	MAT (°C)	MARF (mm)
East Shewa	Adama	1543	8°33′	39°17′	20.80	600
	ATJK	***1642***	7°58′	38°43′	25.00	875
	Bora	***1574***	8°23′	39°00′	20.00	774
	Dugda	***1646***	8°11′	38°44′	21.50	750
	Lume	***1591***	8°34′	39°17′	20.40	969
East Hararghe	Haramaya	***1995***	9°26′	41°30′	16.80	790
	Kersa	***1997***	9°26′	41°57′	16.75	1700

ATJK, Adami-Tulu-Jido-Kombolcha; masl, meter above sea level; MAT, Mean annual temperature; MARF, Mean annual rainfall. Weather data were obtained from District Agriculture Development Office

2.2. Sampling and sample collection

Sampling districts were systematically selected in cooperation with extension staff of the respective Zonal Bureau of Agriculture as they have high potential for onion-production and PB problem. In each district, onion fields were randomly selected at intervals of 1-2 km along the main roads. Within selected fields, 3-5 onion leaves exhibiting typical symptoms of *A. porri* were collected at 10 m points along the diagonals of the field. The samples of each collection were packed in paper envelopes, labeled with the names of the respective locations and date of collection, and taken to Plant Protection Laboratory, School of Plant Sciences, Haramaya University for detection and identification of the pathogen.

2.3. Detection, identification and maintenance of the pathogen

Tissue plating method was employed to isolate the pathogen from the infected leaf samples collected from different locations of Central and Eastern Ethiopia. The infected leaf areas along with some healthy portions were cut into small pieces (1 cm^2) with a sterilized scissor. These pieces were surface-sterilized by dipping in 5% sodium hypochlorite (NaOCl) for three min and rinsed three times with sterile distilled water (SDW) to remove traces of NaOCl. After air dried in a laminar flow cabinet, five pieces were placed on the surface of acidified (2.5 ml of lactic acid L^{-1}) potato dextrose agar (PDA) medium (39 g L^{-1}) in a 90-mm Petri-plate using a flamed forceps. Three replicates were used for each sample. The inoculated Petri-plates were then

incubated in an incubator at 25 ^{o}C for 7-days. Afterwards, fungal colonies developed from the tissue pieces were purified by transferring hyphal-tip sample to fresh PDA plates (Dhingra and Sinclair, 1986) and incubated at 25 ^{o}C for 7-days. Identification of the pathogen isolates was made from pure cultures based on microscopic examination of the specific morphological characters (Ellis, 1971). Pure cultures confirmed to be *A. porri* were transferred to fresh PDA medium slants in culture tubes and incubated at 25 ºC till full growth, then preserved in refrigerator at 4 °C for further use. The typical pure culture and microscopic structures of *A. porri* on PDA medium are shown in Figure 1.

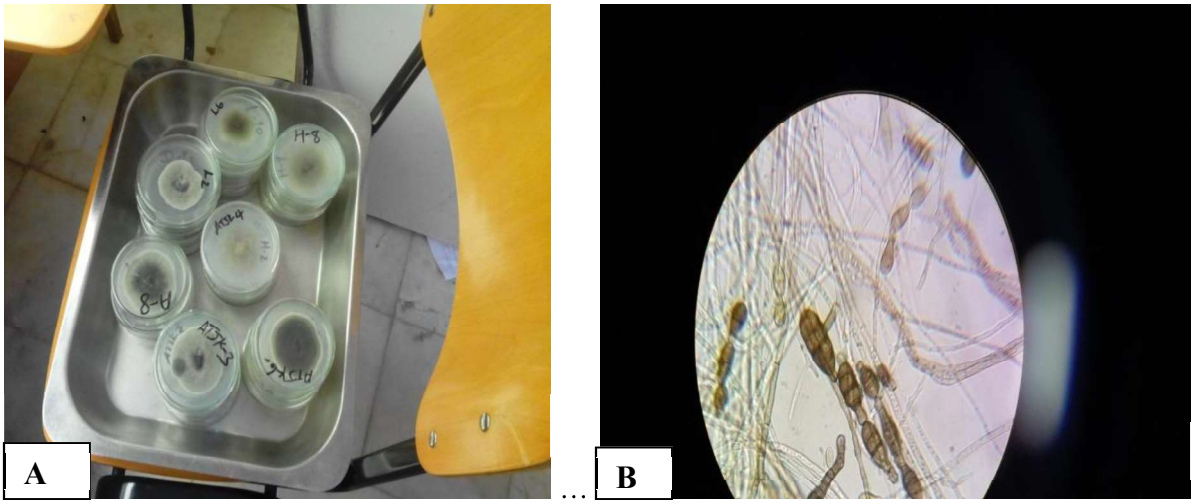

Figure 1: Pure culture (A) and microscopic view (B) of *Alternaria porri* on PDA medium

2.4. Designation of the isolates

The isolates were designated based on their sampling locations (A, Adama; ATJK, Adami-Tulu-Jido-Kombolcha; B, Bora; D, Dugda; H, Haramaya; K, Kersa; L, Lume). A total of 18 *A. porri* isolates (A-1, A-2, A-4, A-5, A-8, ATJK-3, ATJK-6, ATJK-7, B-6, B-7, D-5, D-6, H-2, H-9, K-6, K-8, L-2 and L-10) were randomly taken and used for cultural, morphological and pathological characterization tests as described below.

2.5. Cultural characterization

For cultural characterization, a 6-mm mycelial disc was taken from a week-old actively growing pure culture of each isolate using a sterile cork borer and placed in inverted position in the centre of a 90-mm Petri-dish containing 20-ml of PDA medium. Three replications were maintained for each isolate in a completely randomized design (CRD) and then incubated at 25 ^{o}C (Mohsin *et al*., 2016). Starting from 2-days after incubation, the colony diameter of each isolate was measured (mm) at 24 hr interval as an average of two perpendicular criss-cross diameters until full growth occurred on the Petri-dish (Shahnaz *et al.*, 2013) and a total of six records were made for each isolate. Regression analysis of diameters of colony growth against time after incubations was performed and the slopes were used as measures (mm/day) of colony growth rates for each isolate (Yadav *et al*., 2017). Other colony characters (color, texture, margin and zonation) of the isolates were observed on the culture medium 10-days after incubation. The colony color (front and reverse sides) of each isolate was described using Rayner's Mycological Color Chart (Rayner, 1970). Observations for colony texture, margin and zonation were also described according to Chowdappa *et al*. (2012).

2.6. Morphological characterization

Fifteen-day-old pure cultures of all the isolates were studied for morphological variations in size and septation of conidia as described by Mohsin *et al*. (2016). Slide preparations of the conidial suspensions were made using SDW. Conidial size for each isolate was determined by measuring the length and the width of 30 randomly chosen conidia using an eye-piece (ocular) micrometer (µm) on a calibrated microscope. Number of transverse (horizontal) and longitudinal (vertical) septa in the conidia were counted for the respective isolate.

2.7. Pathological characterization

Pathogenecity test of 18 *A. porri* isolates was conducted on onion seedlings grown in pots under glasshouse conditions. Seed of onion cv. "Adama Red" susceptible to PB was surface-sterilized by dipping in 2% NaOCl for three min, rinsed thrice with SDW to remove traces of NaOCl. After air dried in a laminar flow cabinet, the sterilized seed was sown in plastic seedling trays containing a mixture of sterilized soil, sand and farmyard manure (FYM) at 2:1:1 ratio and maintained in the glasshouse, while watering, weeding and cultivation were performed manually

as deemed necessary. Eight-weeks after sowing, the resulting seedlings (two/pot) were transplanted to 20-cm-diameter plastic pots containing a mixture of air dried soil, sand and FYM at 2:1:1 proportion (Wanggikar *et al*., 2014).

Conidial suspensions of the isolates were prepared using SDW as described by Mohsin *et al*. (2016). Ten ml of SDW was added on 10-days-old pure culture of each isolate and the colony surface was gently rubbed with a sterile glass rod to release conidia. The resulting culture suspension from each isolate was sieved through two-layers of sterile muslin-cloth to remove mycelial fragments, poured into a sterile test tube. One drop of Tween-20 (0.5% v/v) was added to the suspension to maintain uniform conidial dispersion. The concentration of conidia in the suspension was adjusted to $5x10^4$/ml with the help of a haemacytometer under 10 fields of optical microscope. The plants (two/pot) at 5-6 leaf growth stage were inoculated by spraying with conidial suspension ($5x10^4$/ml) of the isolate to run-off (Wanggikar *et al*., 2014), using a sterile atomizer. Non-inoculated control plants were treated with an equal volume of SDW (without inoculum) for comparison. Immediately after inoculation, the plants were covered with polyethylene bags to maintain high relative humidity. The experiment was arranged in a CRD with three replications. The bags were removed after 2-days and plants were kept under normal conditions and high humid condition was maintained by gently spraying SDW using a sterile atomizer (Mohsin *et al*., 2016).

Evaluation of disease severity was started 6-days after inoculation (DAI). The assessment was made every 3-days and a total of six records were made for every pot of each isolate. Severity was recorded on all leaves of the two plants in each pot using 0-5 rating scale (Sharma, 1986), where, 0 = no visible symptom, 1 = a few spots towards the tip, covering up to 10% leaf area (LA), 2 = several dark purplish brown patches, covering 11-20% LA, 3 = several patches with paler outer zone, covering 21-40% LA, 4 = long streaks covering 41-75% LA and 5 = complete drying covering > 75% LA. The severity grades were converted into percentage severity index (PSI) for analysis (Wheeler, 1969):

$$\text{PSI} = \frac{\text{Sum of numerical ratings}}{\text{No. of plants scored x Maximum disease score on scale}} \text{x}100$$

The disease progress rate (DPR) for each isolate was obtained from the regression of PSI values fit to logistic model, ln[(x/1-x)] (Van der Plank, 1963) with dates of assessments, where x is PSI or PB severity index in proportion. The slope of the regression line estimated the DPR. Regression was computed using Minitab software (Release 15.0 for Windows®, 2007). Area under the disease progress curve (AUDPC) (%-days) was also calculated from PSI data assessed at different DAI for each isolate applying the following formula (Madden *et al*., 2008):

$$\text{AUDPC} = \sum_{i=1}^{n-1} 0.5[(x_i + x_{i+1})(t_{i+1} - t_i)]$$

Where, n is the total number of disease assessments, t_i is the time of the i^{th} assessment in days from the first assessment date and x_i is the PSI of disease at the i^{th} assessment. Both the calculated AUDPC and DPR values were used in analysis of variance (ANOVA).

2.8. Data analysis

Data for cultural, morphological and pathogenic characters of the isolates were subjected to ANOVA. The treatment means were separated using Duncan's Multiple Range Test (DMRT) at 5% significance level (Gomez and Gomez, 1984). Simple correlation analysis was used to determine the relationships between cultural and pathological parameters. Both ANOVA and correlation analyses were performed using GLM procedure of the SAS software version 9.1 (SAS Institute, 2003).

3. Results and discussions

3.1. Cultural characterization of A. porri isolates

The isolates significantly ($p \leq 0.001$) differed in colony growth at all dates of assessments after incubation and colony growth rate (CGR) on PDA medium (Table 2). At 7-days after incubation, colony growth of the isolates varied from 49.50-84.00 mm the minimum being for the isolate A-4 and the maximum for the isolate H-9. Pusz (2009) observed that colony growth varied from 48-68 mm among 26 *A. alternata* isolates 7-days after incubation. Goyal *et al.* (2011) also pointed out that variation in colony growth of 13 *A. brassicae* isolates collected from different locations, India. In this study, the mean CGR ranged between 0.29 and 0.66 mm/day the fastest

growth being for the isolate H-2 and K-6, and the least for the isolate A-4. In support of this result, Shahnaz *et al.* (2013) noticed that mean CGR ranged from 0.32-0.92 mm/day among 26 *A. porri* isolates on PDA medium.

Table 2: Variability in colony growth and growth rate (CGR) of 18 *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium incubated at 25 ^{o}C for 7-days

Isolate	Colony growth (mm) at different days after incubation						CGR
	2-days	3-days	4-days	5-days	6-days	7-days	(mm/day)
A-1	17.00^{b-d}	27.83^{d-f}	36.50^{g-j}	43.50^{jk}	48.33^{jk}	52.83^{jk}	0.31^{hi}
A-2	15.17^{d-g}	24.83^{g-i}	34.83^{i-k}	43.67^{i-k}	50.67^{ij}	57.17^{hi}	0.37^{fg}
A-4	16.67^{b-e}	25.50^{g-i}	33.83^{jk}	40.00^{l}	44.77^{l}	49.50^{l}	0.29^{i}
A-5	14.00^{g}	23.50^{i}	32.33^{k}	54.27^{h-k}	53.00^{g-i}	61.77^{e-g}	0.43^{de}
A-8	15.17^{d-g}	27.00^{e-h}	36.00^{h-j}	42.83^{k}	48.27^{jk}	54.27^{i-k}	0.35^{gh}
ATJK-3	16.17^{c-f}	26.00^{f-h}	36.83^{g-i}	46.17^{gh}	53.67^{gh}	61.00^{fg}	0.39^{ef}
ATJK-6	14.00^{g}	25.17^{g-i}	34.83^{i-k}	44.33^{h-k}	54.67^{g}	64.50^{de}	0.45^{d}
ATJK-7	18.00^{bc}	28.83^{c-e}	38.00^{f-h}	45.17^{h-j}	50.50^{ij}	55.77^{h-j}	0.33^{h}
B-6	14.50^{fg}	27.00^{e-h}	37.33^{f-i}	45.77^{g-i}	51.77^{hi}	59.00^{gh}	0.40^{ef}
B-7	15.67^{d-g}	28.17^{c-f}	39.00^{e-g}	47.67^{fg}	55.67^{fg}	66.33^{cd}	0.45^{d}
D-5	18.33^{b}	29.50^{cd}	41.00^{c-e}	49.17^{ef}	57.50^{ef}	63.83^{d-f}	0.40^{ef}
D-6	18.50^{b}	30.33^{bc}	42.17^{c}	52.00^{d}	64.00^{d}	74.50^{b}	0.51^{c}
H-2	14.00^{g}	28.83^{c-e}	41.67^{cd}	54.67^{c}	68.67^{c}	81.00^{a}	0.66^{a}
H-9	22.67^{a}	36.50^{a}	51.17^{a}	66.00^{a}	77.83^{a}	84.00^{a}	0.62^{b}
K-6	18.33^{b}	32.17^{b}	45.67^{b}	58.83^{b}	73.33^{b}	83.67^{a}	0.66^{a}
K-8	16.83^{b-d}	28.50^{c-e}	39.50^{d-f}	50.27^{de}	59.00^{e}	68.50^{c}	0.46^{d}
L-2	14.67^{e-g}	24.67^{hi}	34.83^{i-k}	43.17^{jk}	50.67^{ij}	56.33^{hi}	0.37^{fg}
L-10	15.33^{d-g}	27.17^{d-g}	36.33^{h-j}	42.50^{k}	47.00^{kl}	51.77^{kl}	0.32^{h}
LSD (5%)	0.38^{***}	0.43^{***}	0.48^{***}	0.40^{***}	0.53^{***}	0.64^{***}	0.01^{***}
CV (%)	6.77	4.48	3.70	2.48	2.80	2.97	4.51

Means in the same column followed by the same letter (s) are not significantly different from each other at $p \leq 0.05$ (DMRT); ***, Significant at $p \leq 0.001$.

Apart from the variations in colony growth and CGR, all the isolates exhibited variability in colony color, texture, margin and zonation on PDA medium 10-days after incubation at 25 ^{o}C (Table 3). Front colony color of the isolates varied from mostly dark-gray (38.89%) (Figure 2A) to whitish-gray (5.56%) (Figure 2B). Majority (77.78%) of the isolates had cottony colony texture (Figure 3A), whereas a few (22.22%) isolates produced velvety colony texture (Figure

3B). Colony margin of the isolates was generally irregular (66.67%) (Figure 4A) to circular (33.33%) (Figure 4B). Most (72.22%) of the isolates had zonation (Figure 5A) in their colony and 27.78% of the isolates had no zonation (Figure 5B) in their colony. Undersurfaces (reverse sides) of the colonies varied from mostly (22.23%) reddish-brown (Figure 6A) to dark-gray (5.56%) (Figure 6B) in color. These findings are in agreement with the observations of Chowdappa *et al*. (2012), Shahnaz *et al.* (2013) and Mohsin *et al*. (2016) who reported existence of remarkable variation among onion *A. porri* isolates in their colony color, margin, texture, zonation and pigment production on PDA medium.

Table 3: Variations in colony characteristics of *A. porri* isolates (n = 18) of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C

Isolate	Colony characters				
	Front color	Texture	Margin	Zonation	Reverse color
A-1	Grayish-white	Cottony	Irregular	Present	Greenish-gray
A-2	Grayish-white	Cottony	Irregular	Present	Greenish-gray
A-4	Grayish-white	Cottony	Irregular	Present	Grayish-brown
A-5	Whitish-gray	Cottony	Irregular	Present	Grayish-black
A-8	Grayish-white	Cottony	Irregular	Present	Grayish-brown
ATJK-3	Dark-gray	Cottony	Circular	Present	Grayish-green
ATJK-6	Dark-gray	Velvety	Circular	Present	Grayish-green
ATJK-7	Dark-gray	Cottony	Irregular	Present	Reddish-brown
B-6	Dark-gray	Velvety	Irregular	Present	Reddish-brown
B-7	Dark-green	Velvety	Irregular	Absent	Grayish-black
D-5	Gray	Cottony	Irregular	Present	Brownish-gray
D-6	Gray	Cottony	Irregular	Present	Greenish-black
H-2	Dark-green	Cottony	Circular	Absent	Greenish-black
H-9	Reddish-green	Cottony	Circular	Absent	Reddish-brown
K-6	Reddish-green	Cottony	Circular	Present	Reddish-brown
K-8	Dark-gray	Cottony	Circular	Absent	Brownish-gray
L-2	Dark-gray	Cottony	Irregular	Present	Dark-gray
L-10	Dark-gray	Velvet	Irregular	Absent	Grayish-green

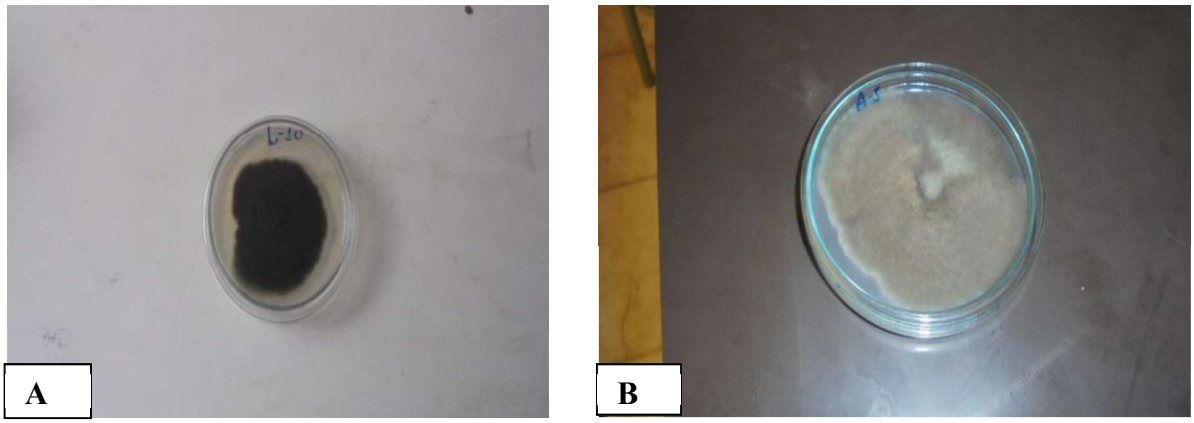

Figure 2: Variability in front colony color of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C: A) Dark-gray; B) Whitish-gray

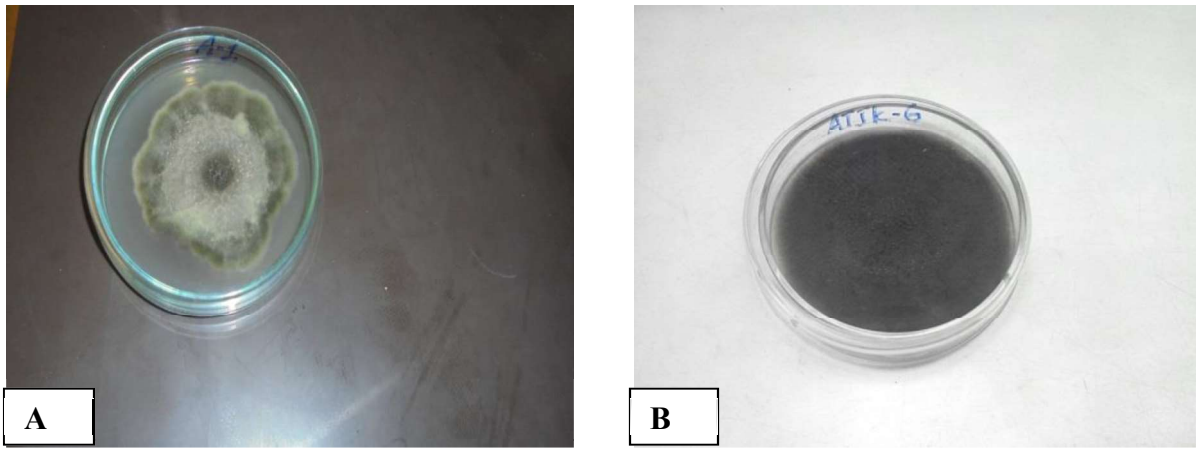

Figure 3: Variability in colony texture of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C: A) Cottony; B) Velvety

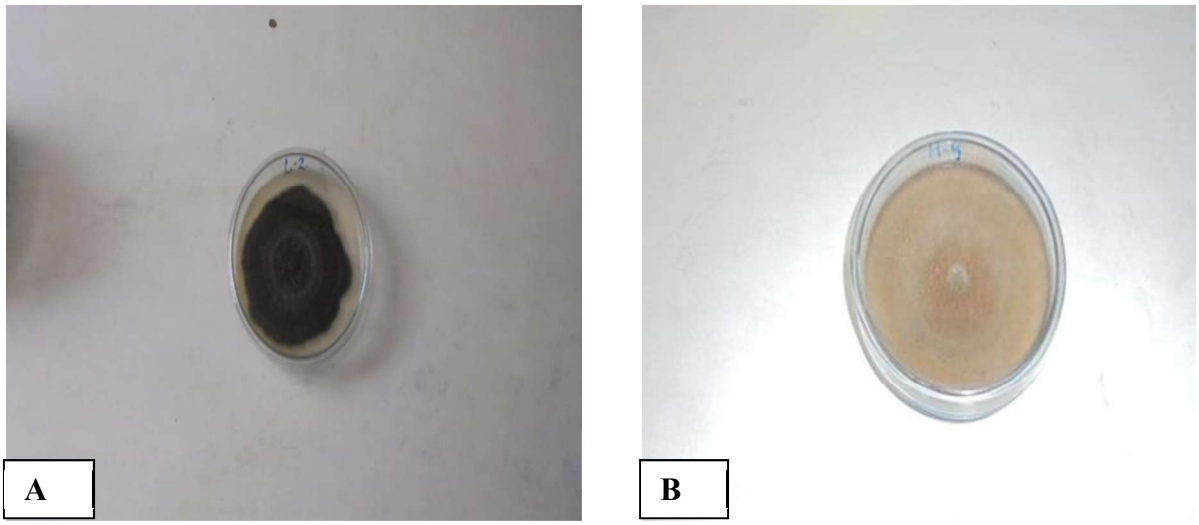

Figure 4: Variability in colony margin of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C: A) Irregular; B) Circular

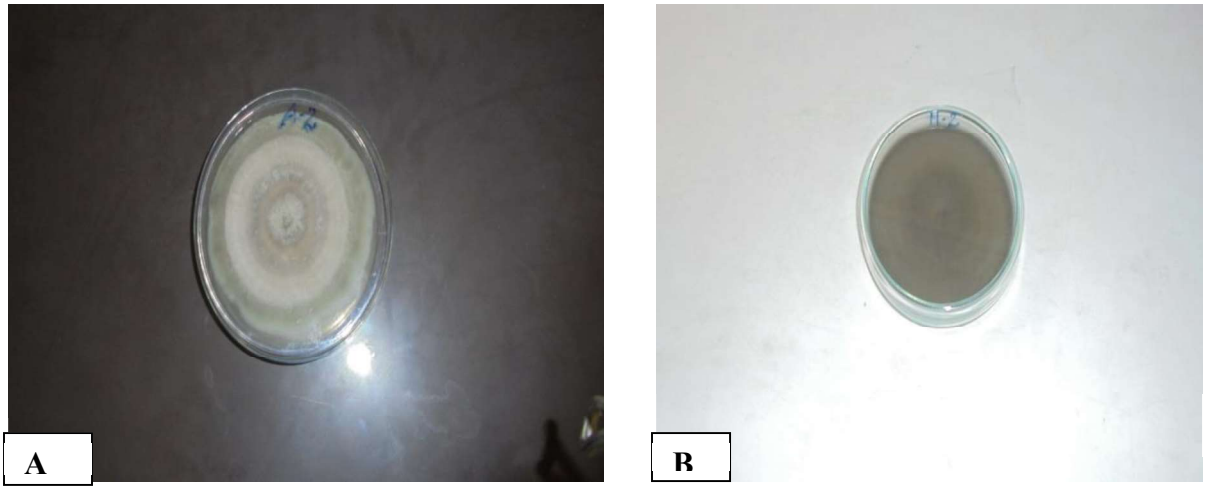

Figure 5: Variability in colony zonation of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C: A) Zonation; B) No zonation

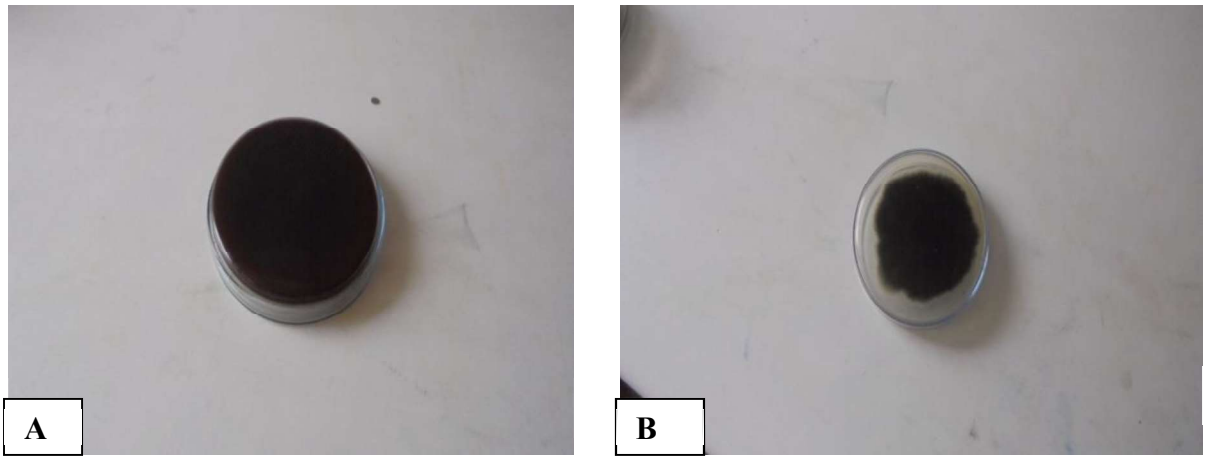

Figure 6: Variability in reverse colony color of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia on PDA medium 10-days after incubation at 25 ^{o}C : A) Reddish-brown; B) Dark-gray

3.2. Morphological characterization of A. porri isolates

All the isolates showed morphological variations in conidial sizes and number of septations (Table 4). The number of transverse and longitudinal septa of conidia varied from 1-6 and 1-3, respectively. The maximum (4.70) mean number of transverse septa was recorded in the isolate L-2 and the minimum (2.07) in isolate A-8. The maximum (2.00) mean number of longitudinal septa was observed in the isolate L-2 and the minimum (1.10) in the isolate A-2. Conidial length of the isolates ranged from 16.50-41.25 µm, while the conidial width ranged from 8.25-13.75 µm. The longest (38.73 µm) and the shortest (19.86 µm) mean conidial lengths were recorded in the isolate H-9 and A-2, respectively, while the widest (12.83 µm) and the narrowest (8.86 µm) mean conidial thicknesses were measured in the isolate H-9 and A-2, respectively. The present findings agree with the observations by Mohsin *et al*. (2016) who reported significant variation in mean conidial size (11.20-39.20 x 4.76-1.43 µm) and number of septations (3-6 transverse and 1-2 longitudinal septa) among 27 *A. porri* isolates associated with onion PB in Bangladesh.

Table 4: Variations in conidial septation and size of *A. porri* isolates (n = 18) of onion purple blotch from Central and Eastern Ethiopia on PDA medium 15-days after incubation at 25 ^{o}C

Isolate	Conidial septation (No.)				Conidial size (µm)			
	Transverse		Longitudinal		Length		Width	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
A-1	2-3	2.33^{d-f}	1-2	1.40^{cd}	25.44-35.75	30.17^{b-d}	10.31-13.75	12.30^{a}
A-2	2-3	2.40^{d-f}	1-2	1.10^{d}	16.50-23.83	19.86^{f}	8.25-9.17	8.86^{b}
A-4	2-3	2.93^{b-e}	1-2	1.30^{cd}	24.75-31.17	28.72^{b-e}	10.31-12.83	11.99^{a}
A-5	2-3	2.40^{d-f}	1-2	1.53^{b-d}	22.46-27.50	25.13^{d-f}	11.18-12.83	12.06^{a}
A-8	1-2	2.07^{f}	1-2	1.40^{cd}	26.58-28.88	27.96^{c-e}	11.69-13.75	12.76^{a}
ATJK-3	2-3	2.80^{b-f}	1-2	1.27^{cd}	27.50-35.75	32.39^{bc}	11.69-13.75	12.76^{a}
ATJK-6	2-4	3.40^{bc}	1-2	1.70^{a-c}	29.33-34.83	32.62^{bc}	11.92-13.75	12.83^{a}
ATJK-7	2-3	2.93^{b-e}	1-2	1.27^{cd}	22.69-24.75	23.57^{ef}	11.00-13.75	12.60^{a}
B-6	2-4	3.00^{b-e}	1-2	1.40^{cd}	20.63-24.75	22.12^{f}	9.63-12.01	10.69^{ab}
B-7	3-5	3.47^{bc}	1-2	1.40^{cd}	29.70-31.17	30.37^{b-d}	11.00-12.83	11.84^{a}
D-5	2-3	2.73^{c-f}	1-2	1.33^{cd}	30.25-37.58	34.83^{ab}	11.00-13.75	12.22^{a}
D-6	2-3	2.27^{ef}	1-2	1.20^{d}	26.81-33.00	29.72^{b-d}	9.72-13.06	11.57^{a}
H-2	3-4	3.40^{bc}	1-2	1.40^{cd}	33.00-36.94	34.41^{ab}	10.18-12.83	11.64^{a}
H-9	3-4	3.60^{b}	1-2	1.93^{ab}	36.99-41.25	38.73^{a}	11.92-13.75	12.83^{a}
K-6	3-4	3.40^{bc}	1-2	1.27^{cd}	33.00-37.13	34.68^{ab}	11.00-13.06	11.99^{a}
K-8	3-4	3.13^{b-d}	1-2	1.53^{b-c}	28.14-33.92	30.31^{b-d}	9.90-12.83	11.24^{a}
L-2	4-6	4.70^{a}	1-3	2.00^{a}	22.92-28.19	24.89^{d-f}	10.08-11.69	10.62^{ab}
L-10	3-4	3.33^{b-c}	1-2	1.30^{cd}	30.80-40.33	34.48^{ab}	11.18-13.75	12.21^{a}
LSD (5%)		0.14***		0.08***		1.07***		0.42*
CV (%)		13.82		15.58		10.62		10.37

Means in the same column followed by the same letter (s) are not significantly different from each other at $p \leq 0.05$ (DMRT); * and ***, Significant at $p \leq 0.05$ and 0.001, respectively.

3.3. Pathological characterization of A. porri isolates

All the tested isolates were able to infect onion seedlings causing typical PB symptoms (Figure 7A). No control seedlings develop PB symptom (Figure 7B). A significant ($p \leq 0.001$) pathogenic variability was observed in disease severity index at all dates of assessments after inoculation, AUDPC and DPR among all the tested isolates (Table 5). At 21-DAI, the highest (89.17%) disease severity index was caused by isolate H-9, whereas the lowest (30.00%) was

recorded from isolate B-7. AUDPC of the isolates varied from 267.20-1068.23%-days with minimum from isolate B-7 and maximum from H-9 isolate. The maximum (0.19 unit/day) mean DPR was also recorded from isolate H-2 and H-9, while the minimum (0.08 unit/day) was recorded from ATJK-3. Jadhav *et al.* (2011) reported the presence of pathological variability among *A. macrospora* isolates causing leaf blight of cotton obtained from different regions. Mohsin *et al*. (2016) also observed varying degrees of virulence among 27 *A. porri* isolates collected from different locations in Bangladesh using a net house infection of onion leaf assay. The pathogenic variability of the isolates was partially matched with colony growth rate on PDA medium. The highly pathogenic isolates showed more colony growth rate on PDA medium than the other isolates. This finding is in contradictory with that of the findings of Dam *et al*. (2010) who reported that the isolates of *A. alternata* exhibiting higher degree of virulence had slower colony growth rate than the less virulent isolates.

Table 5: Variability in disease severity index, AUDPC and DRR among 18 *A. porri* isolates from Central and Eastern Ethiopia on onion seedlings (cv., Adama Red) under glasshouse conditions

Isolate	Disease severity index (%) at different days after inoculation						AUDPC	DPR
	6-days	9-days	12-days	15-days	18-days	21-days	(%-days)	(Unit/day)
A-1	10.83^{c}	18.33^{de}	22.22^{e-g}	23.89^{d-f}	37.50^{bc}	45.83^{c}	390.83^{e-g}	0.13^{bc}
A-2	12.50^{c}	15.00^{d-f}	17.50^{fg}	17.50^{f}	26.67^{e}	35.00^{f-i}	301.25^{hi}	0.09^{de}
A-4	13..33^{c}	16.67^{d-f}	24.44^{de}	28.33^{d}	38.33^{bc}	54.17^{b}	424.59^{e}	0.14^{bc}
A-5	12.78^{c}	17.78^{d-f}	23.33^{de-f}	26.67^{de}	37.78^{bc}	44.17^{cd}	402.08^{ef}	0.11^{cd}
A-8	10.83^{c}	14.17^{ef}	16.67^{g}	16.67^{f}	25.00^{e}	32.50^{g-i}	282.50^{i}	0.09^{de}
ATJK-3	11.67^{c}	12.78^{f}	17.22^{g}	18.33^{f}	22.78^{e}	31.11^{hi}	277.50^{i}	0.08^{e}
ATJK-6	11.67^{c}	14.44^{ef}	17.22^{g}	19.44^{f}	27.22^{e}	35.56^{f-i}	305.83^{hi}	0.10^{de}
ATJK-7	12.78^{c}	20.00^{d}	28.33^{d}	36.67^{c}	43.33^{b}	56.11^{b}	488.34^{d}	0.14^{b}
B-6	11.67^{c}	13.89^{ef}	16.67^{g}	20.56^{ef}	29.17^{de}	40.83^{c-f}	319.58^{hi}	0.11^{c-e}
B-7	10.56^{c}	14.34^{ef}	16.11^{g}	16.67^{f}	21.67^{e}	30.00^{i}	267.20^{i}	0.09^{de}
D-5	11.67^{c}	16.11^{d-f}	18.33^{fg}	20.56^{ef}	35.83^{cd}	42.50^{c-e}	353.75^{f-h}	0.12^{cd}
D-6	12.78^{c}	16.67^{d-f}	19.17^{e-g}	22.50^{d-f}	27.50^{de}	38.89^{d-g}	335.00^{g-i}	0.10^{de}
H-2	25.00^{b}	33.33^{c}	45.83^{c}	68.33^{b}	78.33^{a}	84.44^{a}	841.67^{c}	0.19^{a}
H-9	31.88^{a}	62.50^{a}	72.50^{a}	77.78^{a}	82.78^{a}	89.17^{a}	1068.23^{a}	0.19^{a}
K-6	27.50^{b}	49.17^{b}	65.00^{b}	76.67^{a}	82.09^{a}	84.17^{a}	986.26^{b}	0.18^{a}
K-8	13.33^{c}	16.25^{d-f}	18.33^{fg}	20.56^{ef}	29.17^{de}	37.50^{e-h}	330.00^{g-i}	0.09^{de}

L-2	12.22^{c}	16.311^{d-f}	17.22^{g}	18.89^{f}	26.67^{e}	35.56^{f-i}	308.33^{hi}	0.09^{de}
L-10	11.66^{c}	16.11^{d-f}	19.44^{e-g}	19.44^{f}	28.33^{e}	35.00^{f-i}	319.99^{hi}	0.09^{de}
LSD (5%)	0.66***	0.89***	1.06***	1.28***	1.37***	1.16***	12.45***	0.01***
CV (%)	13.14	12.29	11.83	12.28	10.33	7.19	8.23	13.28

Means in the same column followed by the same letter (s) are not significantly different from each other at $p \leq 0.05$ (DMRT); ***, Significant at $p \leq 0.001$.

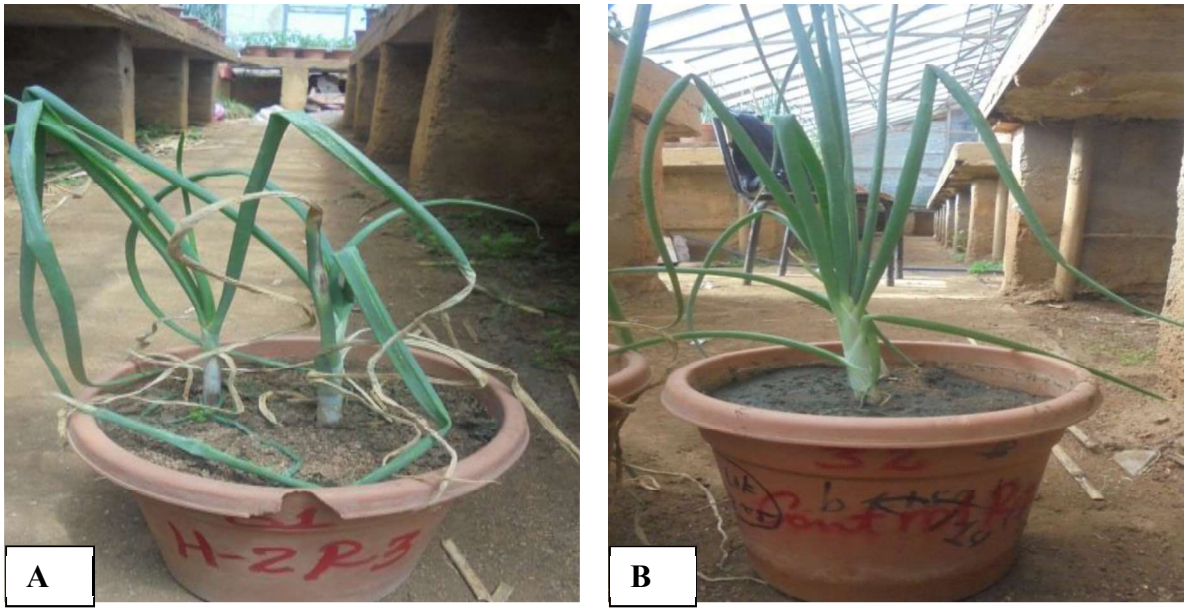

Figure 7: Pathogenecity test of *A. porri* from Central and Eastern Ethiopia on onion seedlings (cv., Adama Red) under glasshouse conditions: A) Inoculated plants; B) Control plants

3.4. Correlation analysis

Simple correlation analysis revealed that final colony growth, growth rate and conidial length had positive and highly significant correlations with final disease severity index, AUDPC and DPR (Table 6). In general, final colony growth and growth rate had higher (r is near to one) positive correlations with final disease severity index, AUDPC and DPR.

Table 6: Correlation coefficient (r) between cultural and pathological parameters of *A. porri* isolates of onion purple blotch from Central and Eastern Ethiopia, 2016

Parameter	Correlation coefficients (r)					
	FCG	CGR	CL	FDSI	AUDPC	DPR
FCG	1					
CGR	0.9772***	1				
CL	0.5086***	0.4755***	1			
FDSI	0.6727***	0.7019***	0.4131***	1		
AUDPC	0.7418***	0.7664***	0.4697***	0.9734***	1	
DPR	0.5390***	0.5682***	0.3404**	0.9515***	0.8938***	1

FCG, Final colony growth; CGR, Colony growth rate; CL, Conidial length; FDSI, Final disease severity index; AUDPC, Area under the disease progress curve; DPR, disease progress rate; ** and ***, significant levels at $p \leq 0.001$ and 0.0001, respectively.

In the current study, the cultural, morphological and pathological characters observed on the 18 isolates were not according to the geographical locations of the isolates. In support of this argument, earlier researchers had reported the variability in *A. porri* isolates of onion PB were not according to the geographical locations from where the isolates were collected (Chowdappa *et al*., 2012; Mohsin *et al*., 2016). The changes occurring within the pathogen population might have been because of strong selection pressure resulting from widespread use of mono-cultured crops with little to no genetic diversity and indiscriminate application of synthetic fungicides for long period of time (Weber and Halterman, 2012; Sofi *et al*., 2013).

4. Conclusions and recommendations

The results indicated that *A. porri* isolates of onion PB in Central and Eastern regions of Ethiopia were highly variable in cultural and morphological characteristics, and pathogenic potential aspects regardless of their origin. Availability of high level of variability in population of *A. porri* may be one of the main causes of absence of sufficiently effective onion PB control measures. The results obtained from present research work could be considered for development of sound and reliable management of onion PB through various cultural, varietal, biological, and chemical means. The most aggressive isolate, H-9 identified in this study may be used further to evaluate the effectiveness of possible onion PB management practices in the areas. Moreover, extensive variability studies with inclusion of molecular characterization are necessary to

recognize the overall nature of the Ethiopian *A. porri* isolates and focus efforts in developing an integrated and sustainable PB management system (s) for potential onion-growing regions in the country and elsewhere with similar agro-ecological settings.

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