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CHARACTERIZATION OF FUNGAL PATHOGENS OF YAM (*DIOSCOREA* SP.) FROM SEKA CHEKORESSA DISTRICT, JIMMA ZONE, SOUTHWESTERN ETHIOPIA

Bikila Wedajo[1] and Tesfaye Alemu[2,*]

ABSTRACT: In the present study, characterization of different fungal pathogens (*Fusarium* isolate (AUF1), *Alternaria* isolates (AUA1 and AUA2) and *Verticillium* isolates (AUV1 and AUV2) was done from infected yam leaves and tubers grown in Seka Chekoressa district, Jimma Zone, southwestern Ethiopia. The fungal isolates were identified to the genus level based on cultural and microscopic characteristics. Pathogenicity test was performed by inoculating apparently healthy yam leaves and tubers of which *Fusarium* AUF1 strain, *Alternaria* AUA1 strain and *Verticillium* AUV1 strain were the most virulent ones with 100% infection of leaves and tubers evident from the development of disease symptoms. Among the isolates, *Fusarium* AUF1 and *Verticillium* AUV1 displayed the maximum mycelial growth diameter of 90 mm on potato dextrose agar medium (PDA). All fungal pathogens showed maximum growth at 25°C with the exception of *Alternaria* AUA1 that achieved the same growth at 30°C. With regard to pH, the maximum dry mycelial weight was recorded at pH 6.0 from *Alternaria* AUF1 (624.5±4.0 mg), *Alternaria* AUA1 (693.9±3.3 mg) and *Verticillium* AUV2 (697.6±1.6 mg). However, *Alternaria* AUA2 and *Verticillium* AUV1 gave maximum dry mycelial weight of 356.4±1.1 mg and 693.4±4.9 mg at pH 7.0 and 5.0, respectively. Likewise, *Fusarium* AUF1 and *Verticillium* AUV1 grew best on dextrose containing medium; whereas *Alternaria* AUA1, *Alternaria* AUA2 and *Verticillium* AUV2 preferred maltose as the best carbon source for growth. Potassium nitrate was the best nitrogen source for all fungal isolates with the exception of *Alternaria* AUA2 which grew better on ammonium nitrate.

Key words/phrases: *Dioscorea* species, Fungal isolates, Growth parameters, *In vitro*.

[1] Department of Biology, College of Natural Sciences, Arba Minch University, P.O. Box 21, Arba Minch, Ethiopia. E-mail: bikilawedajo@yahoo.com
[2] Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: tesfayealemu932@gmail.com; tesfaye.alemu@aau.edu.et
*Author to whom all correspondence should be addressed

INTRODUCTION

Yams (*Dioscorea* spp.) constitute among the most economically important staple foods for millions of people in the world, especially in some parts of the tropics and subtropics for it contains the highest protein content of all root and tuber crops (Okigbo and Ogbonnaya, 2006). West Africa accounts for over 90% of the 4.59 million hectares of yam cultivation worldwide with production of 20–25 million tons per year, with a superior economic significance compared to other crops at the regional level (Izekor and Olumese, 2010; Sanginga and Mbabu, 2015). Ethiopia and Sudan are the major yam producing countries in East Africa (Musa *et al*., 2011). In Ethiopia, yam plants are mostly distributed in South and southwestern of Ethiopia including Metekel, Sidama, Wolayta, Gambella, Bench Majii, Jimma and North Omo (Umeta *et al*., 2005).

There are several limiting factors for the production of yam in the world. Yam plants are susceptible to infection by fungi, bacteria and viruses at all stages of growth and during storage. Tuber rot is a major factor that limits the post-harvest life of yams and yield losses can be very high which is estimated at about 26% in the world (Amusa *et al*., 2003). Most rots of yam tubers in the storage are caused by pathogenic fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solani*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Rhizoctonia* spp., *Penicillium oxalicum* and *Rhizopus nodosus* (Okigbo and Ikediugwu, 2002; Okigbo and Emoghene, 2004; Suleiman, 2010). In Ghana, it was observed that up to 70% of yam plants from farmers' fields and stored yam were affected by fungi (Aidoo, 2007).

The most important field pathogen is the foliar anthracnose (*Colletotrichum gloeosporioides*) which is a major threat to yam cultivation, in all yam producing areas (Abang *et al*., 2003). Anthracnose can affect all parts of the yam plant at all stages of crop development (Akem, 1999). The disease causes leaf necrosis and shoot dieback of yams, thus reducing the photosynthetic efficiency of the plant, which results in yield losses of over 90% in susceptible genotypes (Egesi *et al*., 2007). Many fungal pathogens of yam are known in many countries, but in Ethiopia although leaves and tubers of yam plants are infected by various fungal pathogens in the major yam growing areas of Ethiopia, there is limited information on the diversity of the pathogens.

It is clearly observed that there is high disease incidence and infection on yam plants in Seka Chekoressa district, Jimma Zone, Ethiopia. The

objective of this study was to isolate and characterize the causal pathogens of yam crop from these areas on different culture media, carbon and nitrogen sources at different pH and incubation temperature under laboratory condition.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Seka Chekoressa district, Jimma Zone of Oromia Region, Ethiopia. The Jimma Zone is located 363 km southwest of Addis Ababa. Jimma is situated at 1710 m above sea level, 36°37′ E longitude and 7°55′ N altitude. The minimum temperature is 11.8°C and the maximum temperature was 28°C. The rainfall averages about 1529.5 mm average per year. There was an extended period of rains for about eight months. The seasonal distribution of rainfall was 17.5% in cool dry season (October to February), 56.3% in the rainy season (June to September) and 26.2% in early rains (March, April and May). The average relative humidity amounted to 68%. Its agro-ecology is sub-humid tepid to cool mid highlands. Major soil types are upland with chromic nitosol and combisol, and bottom land with fluvisol.

Experimental site and sample collection

All the experiments were carried out at the Mycological Research Laboratory, Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University. Diseased samples of yam were collected from Seka Chekoressa District, Jimma Zone, Ethiopia. The different parts of the plant samples (tubers and leaves) were brought to Mycological Laboratory in clean plastic bags (envelopes). The samples were kept in a refrigerator at +4°C until isolation for further investigation.

Isolation of fungal pathogens

The diseased leaves and tubers were cut into 1 cm size from the periphery of the infected rotten parts with a sterilized knife and washed in sterilized distilled water for two minutes on separate plates. The samples were subsequently dipped in 70% ethanol for one minute to sterilize the surface and rinsed three times with sterile distilled water. The samples were then allowed to dry with a sterile filter paper in laminar flow chamber. Five dried pieces per plate were placed on a PDA medium. The Petri-plates were incubated at 25°C±1 and observed periodically for the growth of fungal colonies. Representative fungal colonies from plated tissues were selected

and purified through repeated sub-culturing onto PDA which contained chloramphenicol at 2 mg/ml (Aneja, 2005). The pure colonies were transferred on to PDA slants for further study.

Identification of fungal isolates

The fungal isolates from infected yam tubers and leaves such as pigment production, colony colour, spore or conidia producing structures and spore shapes were recorded. Spore and mycelium characteristics were studied using the compound microscope 40x and 100x magnification (LABOMED, New York). These characteristics were used in identifying the fungal isolates to the genus level following standard methods described by Mathur and Kongsdal (2003) and Barnett and Hunter (1972). A total of ten fungal isolates were identified as pathogens of yam. Among these isolates, only five isolates were selected for this study based on their pathogenicity test and virulent characters.

Designation of isolates

Fungal isolates that were found to be pathogenic were designated by the prefix AU (Addis Ababa University), letters F (*Fusarium*), A (*Alternaria*) and V (*Verticillium*) followed by specific Arabic numbers to separate the different types of fungal isolates.

Preparation of inoculum and pathogenicity test of isolates

The fungal isolates were prepared by growing them on sterilized potato dextrose broth medium (PDB) in 250 ml conical flask containing 100 ml of the broth and incubated for 10 days at 25°C. The mycelial mats were filtered using Whatman No. 42 filter papers and thoroughly washed. The harvested mycelial mats were mixed in a blender and the required amount of water (10 ml) was added to get the 10 ml volume of the inoculum. The inoculum, therefore, consisted of mycelium bits and conidia. However, spore concentration in the blended material was counted in each case by using a haemocytometer and the concentration of spore suspensions were adjusted to 1×10^6 conidia/ml (Aneja, 2005).

Healthy-looking yam tubers and leaves were washed with distilled water and surface sterilized with 70% ethanol and subsequently rinsed with sterilized water. Different Petri-plates with filter paper and slides were sterilized. The sterilized yam tubers and leaves were well dried in laminar air flow hood and cut into 1 cm square pieces. A small amount of sterile distilled water was added to each Petri-plate to make the environment moist and the pieces of the cut plants were put onto the slides. The samples were

inoculated with two 2 ml of the standardized spore suspension. The isolates were incubated at 25±1°C for 20 days. The inoculated leaves and tubers were examined daily for evidence of fungal growth, yam lesions, colonization, discolouration and mortality as well as other symptoms. The controls were inoculated with 2 ml sterile distilled water. Triplicates were maintained for each treatment. The re-isolation was undertaken from infected leaves and tubers using standard methods as before and compared with the original culture. Measurement of pathogenicity was shown as scaling percent of tissues infections, and sporulation under the microscopic field (100x) according to the techniques described by Okafor (1966).

Preparation of slide culture for isolates

The slide culture apparatus was prepared on sterile Petri-dish inside which a slide was placed on a glass rod. Aseptically, with the help of sterile forceps, water soaked cotton was placed in a sterilized Petri-dish to completely moisten it. With the help of a sterile scalpel, a 5 mm fungal agar block from actively growing region was cut from the plate of PDA medium. The block of agar medium was picked up by inserting the scalpel and carefully transferred to the centre of the slide under aseptic condition. Mycelial fragments and spores were taken from the fungus culture and inoculated in to the four sides of the agar square blocks on which the fungal isolates were grown on PDA for one week or more, depending on the fungal isolates. Sterile cover slips were placed on the upper surface of the agar block. Thereafter, the Petri-dishes were incubated at 25°C for twenty days. After twenty days, cover slips were carefully removed and put on a clean slide with a drop of lactophenol cotton blue. Likewise, the agar block was carefully removed from the previous slide and covered with cover slip after having a drop of lactophenol cotton blue. The slides were observed under low power (10x), middle (40x) power and high power objective (100x) using ocular and stage micrometer. The average measurements of spore were determined and shapes of the spores were recorded using Olympus microscope (OLYMPUS, Japanese). Microphotographs were taken to show the typical spore morphology of the fungal isolates (Sangdee *et al*., 2011; Getachew Gashaw *et al*., 2013).

Cultural and morphological characterization

Four different solid media; Potato Dextrose Agar (PDA; Oxoid), Malt Extract Agar (MEA; Oxoid), Czapeck Dox's agar (CDA; Oxoid) and Sabouraud's Dextrose Agar (SDA; Oxoid) were used for the study of growth characters. Each culture medium was prepared in one litre of

distilled water as per the manufacturers' recommendation and autoclaved at 121°C for 15 minutes. Twenty five millilitre of each media was poured aseptically into Petri-dishes of 90 mm diameter. Five mm discs from an actively growing zone of seven days old fungal culture were placed upside down at the centre of the solidified medium and were incubated at 25±1°C. Three replications were maintained for each media tested (Mathur *et al*., 1950). The radial measurements of the colony growth were taken ten days after inoculation when the maximum growth was attained in any one of the media tested. Photographs were taken by using camera to show the growth behavior of the fungal isolates. A compound microscope with low (10x), middle (40x) and high (100x) magnification power was used (Barnett and Hunter, 1972).

Eco-physiological studies of fungal isolates

Effect of different temperature on mycelial growth of fungal isolates

During this investigation, the PDA medium was used to study the effect of different temperature requirement on the mycelial growth of the test pathogens. Mycelial disc of 5 mm diameter of each isolate was inoculated into Petri-plates containing PDA medium and incubated at 20°C, 25°C, 30ºC, 35°C and 40°C for eight days. Each experiment was done in three replications. The mycelial growth diameter was measured in millimetre for each treatment (Ramteke and Kamble, 2011; Getachew Gashaw *et al*., 2013).

Effect of hydrogen ion concentration on mycelial growth of fungal isolates

The effect of pH on the growth of fungal isolates was also tested under *in vitro* condition using liquid cultures containing different pH levels. Potato dextrose broth (PDB) medium was used to study the effect of pH of the medium on the mycelial growth of different fungal isolates. Hundred millilitres of liquid PDB medium was dispensed into a 250 ml conical flask under aseptic conditions. The pH levels of the broth medium adjusted to 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 were used for this study using 1N NaOH and 1N HCl (Naik *et al*., 1988). Each flask was inoculated with fungal isolates using 5 mm diameter mycelial disc under aseptic conditions by using sterilized cork borer. Inoculated flasks were incubated at 25±1°C for fifteen days and the dry mycelial weights were measured. The cultures were filtered through Whatman No. 42 filter paper and the mycelial mats were dried at 45°C for 48 h. The dry mycelial weight was measured by subtracting the initial

weight of the filter paper from the weight of the filter paper along with the mycelial mat. Two replications were maintained for each treatment (Getachew Gashaw *et al*., 2013).

Nutritional studies of fungal isolates

The effect of carbon sources on mycelial growth of fungal isolates

For this study, the following carbon sources were selected randomly and incorporated into Richard's agar medium which was taken as basal medium that contained dextrose, sucrose, maltose and fructose (Otsuka *et al*., 1957). Richard's agar medium consisted of KNO_3 10 g, KH_2PO_4 5 g, $MgSO_4$ 2.5 g, $FeCl_3$ 0.02 g, Sucrose 50 g and Agar-Agar 20 g (Lilly and Barnett, 1951). For this study, sucrose was replaced by equivalent amount of the above mentioned carbon sources individually. Carbon sources were added to Richard's agar medium at 21.053 g of carbon per litre of autoclaved medium. Thereafter, each conical flask containing Richard's agar medium were poured into Petri-plates (25 ml per plate) under aseptic condition and cooled. After solidification of the medium, 5 mm diameter agar plugs growing on PDA were cut from one week actively growing culture of the fungus with the help of sterilized cork borer, placed in the centre of each Petri-plate and incubated for seven days at 25±1°C. Three replicates were used for each carbon source per isolate. Mycelial growth diameter of the fungal isolates was measured using a ruler after seven days (Getachew Gashaw *et al*., 2013).

Effect of nitrogen sources on mycelial growth of fungal isolates

Nitrogen sources were evaluated using Richard's agar medium. The potassium nitrate in Richard's medium was replaced by ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3) and urea (CH_4N_2O) by considering potassium nitrate (KNO_3) as one treatment. The amount of nitrogen in Richard's agar medium was replaced by equivalent amount of the nitrogen sources (1.3855 g) individually in sterile Richard's agar medium. Thereafter, each conical flask containing Richard's agar medium were poured into Petri-plates (25 ml per plate) under aseptic condition and cooled. After solidification of the medium, 5 mm diameter agar plugs growing on PDA were cut from one week actively growing culture of the fungus with the help of sterilized cork borer, placed at the centre of each Petri-plate and incubated for seven days at 25±1°C. For each treatment, three replications were maintained for each nitrogen source per fungal test pathogen. Mycelial growth diameter of the fungal isolates was measured

after seven days (Getachew Gashaw *et al*., 2013).

Data analysis

The mycelial growth diameters of fungal isolates on different media, temperature, pH, carbon and nitrogen sources were analysed for significant differences. Analysis of different parameters was conducted using SPSS statistical analysis software version 16. Mean separation was determined according to Duncan's multiple range test ($p<0.05$).

RESULTS

Isolation and identification of fungal isolates of yam

In the present study, ten different fungal isolates were isolated from infected leaves and tubers of yam from Seka Chekoressa district, Jimma Zone, Ethiopia. Among these isolates, only five isolates were identified as causal pathogens of yam (Fig. 1). These isolates were tested and confirmed that the fungal isolates were pathogens of yam based on pathogenicity test results that were observed on the yam (Table 1). These fungal isolates were morphologically identified as *Fusarium* spp. (AUF1), *Alternaria* spp. (AUA1 and AUA2) and *Verticillium* spp. (AUV1 and AUV2) (Fig. 1).

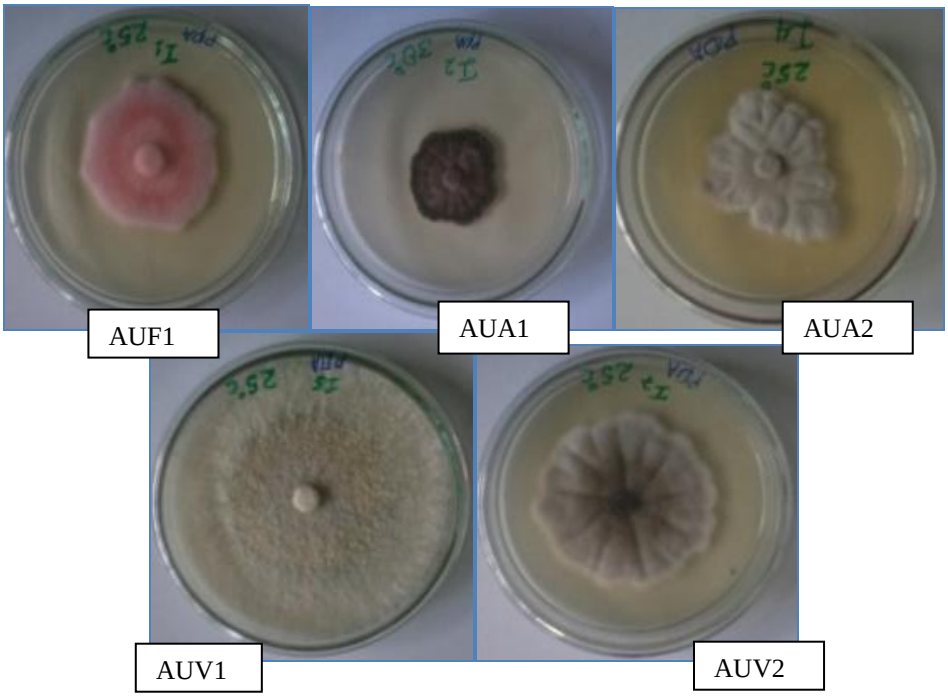

Fig. 1. Culture characteristics of fungal isolates recovered and identified from diseased yam.

Pathogenicity test

The pathogenicity test showed that all the five fungal isolates (AUF1, AUA1, AUA2, AUV1 and AUV2) had confirmed pathogenic virulent character and hence, the fungal isolates showed rot lesions, discolouration, colonization and mortality on healthy looking yam leaves and tubers after twenty (20) days of inoculation (Table 1). The most virulent among the five test fungal isolates were AUF1, AUA1 and AUV1 with tissue infection of 100%, followed by AUV2 with tissue infection of 75%, while the least virulent was AUA2 with tissue infection of 55% (Table 1). The re-isolated fungal isolates were similar with that of the original isolates recovered from infected leaves and tubers culturally and morphologically.

Table 1. Per cent of tissue infections and sporulation on yam leaves and tubers by fungal isolates.

Fungal isolates	Per cent of tissue infection	The value of pathogenicity	Sporulation
AUF1	100	+++	Abundant
AUA1	100	+++	Abundant
AUA2	55	+	Scanty
AUV1	100	+++	Abundant
AUV2	75	++	Moderate

Note: 25–50= scanty under microscopic field (100x), 51–75= moderate under microscopic field (100x), 76–100= abundant under microscopic field (100x)

+=poorly pathogenic (means the fungal isolate simply revealed a sort of growth on the tested yam plant leaves and tubers), ++=moderately pathogenic (due to fungal growth, discolouration of tested yam leaves and tubers occurred), +++= highly pathogenic (tested yam leaves and tubers were highly colonized resulting in rot lesions and mortality of yam plant)

Characterization of the fungal isolates

Cultural characteristics

With regards to colony colour, the fungal isolates showed pink (AUF1), dark gray (AUA1), gray (AUA2), white (AUV1 and AUV2) on the front sides and black colour on reverse sides of all growth media with mycelial topography of flat and raised fluffy growth, except AUF1 that showed white colour on both sides of the CDA medium (Table 2). Isolates AUF1, AUA1 and AUA2 showed raised fluffy growth, whereas AUV1 and AUA2 showed flat mycelium growth on all the media (Table 2). There was no significant difference on pigmentation of AUA1 and AUA2 with dark gray and gray on the front sides, while black colour was exhibited on the reverse sides of all the media, respectively (Table 2). AUV1 and AUV2 revealed white colour on the front side and black colour on the reverse side of all the media tested (Table 2).

Table 2. Effect of different culture media on growth of fungal isolates.

Type of media	Fungal isolates	Cultural characteristics of fungal isolates		
		Front side	**Reverse side**	**Topography of mycelium**
PDA	AUF1	Pink	Pink	Raised fluffy growth
	AUA1	Dark gray	Black	Raised fluffy growth
	AUA2	Gray	Black	Raised fluffy growth
	AUV1	Whitish	Black	Flat mycelium growth
	AUV2	White	Black	Flat mycelium growth
MEA	AUF1	Pink	Pink	Raised fluffy growth
	AUA1	Dark gray	Black	Raised fluffy growth
	AUA2	Gray	Black	Raised fluffy growth
	AUV1	White	Black	Flat mycelium growth
	AUV2	White	Black	Flat mycelium growth
CDA	AUF1	White	White	Raised fluffy growth
	AUA1	Dark gray	Black	Raised fluffy at center
	AUA2	Gray	Black	Raised fluffy growth
	AUV1	White	Black	Flat mycelium growth
	AUV2	White	Black	Flat mycelium growth
SDA	AUF1	Pink	Pink	Raised fluffy growth
	AUA1	Dark gray	Black	Raised fluffy growth
	AUA2	Gray	Black	Raised fluffy growth
	AUV1	White	Black	Flat mycelium growth
	AUV2	White	Black	Flat mycelium growth

The effect of the different types of media on the growth of the fungal isolates revealed that all of them supported the growth of all the isolates (Table 3). AUF1 (90.00±0.00 mm) and AUV1 (90.00±0.00 mm) showed the highest mycelial growth diameter on PDA medium followed by AUA1 (88.67±1.53 mm), AUV2 (75.83±3.01 mm) and AUA2 (65.00±0.00 mm). Likewise, the result revealed that the maximum mycelial growth diameter (85.67±1.53 mm on CDA and 66.33±2.08 mm on SDA medium) was measured by AUF1. However, minimum mycelial growth diameter (61.33±3.21 mm) was exhibited by AUA2 on MEA medium (Table 3). On the other hand, the lowest mycelial growth diameter of 56.33±1.52 mm (AUA2) and 42.67±2.08 mm (AUV1) on CDA and SDA was recorded, respectively (Table 3). The means of mycelial growth diameter of the isolates were significantly ($p<0.05$) different.

Table 3. The effect of different solid media on mycelial growth of fungal isolates after 10 days of incubation at 25°C.

Fungal isolates	Mean mycelial growth diameter in mm (Mean±SD)			
	PDA	MEA	CDA	SDA
AUF1	90.00±0.00a	90.00±0.00a	85.67±1.53a	66.33±2.08a
AUA1	88.67±1.53a	65.67±1.53c	61.33±3.21c	55.00±2.00b
AUA2	65.00±0.00d	61.33±3.21d	56.33±1.52d	50.33±1.52c
AUV1	90.00±0.00a	80.33±1.53b	72.66±2.51b	42.67±2.08d
AUV2	75.83±3.01c	69.00±1.00c	65.67±3.51c	42.83±2.25d
Mean±SD	81.9±10.42b	73.27±10.94c	68.33±10.77b	51.43±9.26c

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly different ($p<0.05$) according to Duncan's multiple range test.

Conidial characteristics

The ranges of conidial lengths of fungal isolates were 19.0–26.2 µm, 30.5–40.47 µm, 27.3–42.5 µm, 3.5–11.2 µm and 2.5–9.0 µm for AUF1, AUA1, AUA2, AUV1 and AUV2, respectively (Table 4 and Fig. 2). In the same pattern, the range of conidial width of AUF1 was 3.2–5.8 µm, AUA1 (10.9–17.37 µm), AUA2 (11.6–16.9 µm), AUV1 (1.8–3.8 µm) and AUV2 (1.25–3.2 µm). The spores of *Fusarium* sp. (AUF1) had banana-shaped conidia with foot cells in sporodochia and three septate spores were commonly found (Table 4). Likewise, the conidia of *Alternaria* spp. (AUA1 and AUA2) were of muriform shape and had light brown colour. The conidial shapes of *Verticillium* spp. (AUV1 and AUV2) were hyaline, ovoid-ellipsoidal to sub-cylindrical shape produced at the apices of phialides (Table 4).

Table 4. Conidial size (length and width) and other features of fungal isolates of yam after twenty days of incubation at 25°C.

Fungal isolates	Length (µm)		Width (µm)		Conidial features		Genus name
	Range	Mean±SD	Range	Mean±SD	Shape	Septation	
AUF1	19.0–26.2	22.07±2.6b	3.2–5.8	4.56±1.1b	Banana	3–5	*Fusarium* sp.
AUA1	30.5–40.47	36.22±4.1a	10.9–17.37	13.84±2.5a	Muriform	5–7	*Alternaria* sp.
AUA2	27.3–42.5	36.41±7.1a	11.6–16.9	14.44±2.1a	Muriform	5–7	*Alternaria* sp.
AUV1	3.5–11.2	7.73±3.4c	1.8–3.8	2.56±0.8b	Hyaline	1	*Verticillium* sp.
AUV2	2.5–9.0	5.79±2.7c	1.25–3.2	2.31±0.8b	Hyaline	1	*Verticillium* sp.

Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test.

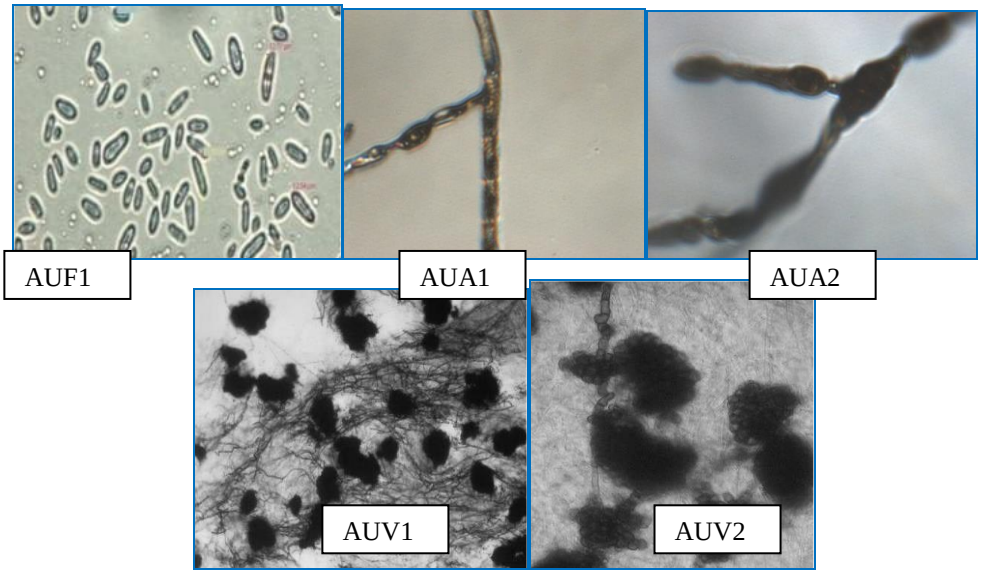

Fig. 2. Conidial morphology of fungal isolates when slide culture was observed under microscope.

The effect of different temperature on the mycelial growth of fungal isolates

The different temperatures tested had an effect on the growth of fungal isolates (Table 5). The mean colony diameter of all the fungal isolates on solid medium was maximum within a temperature range of 25–30°C (Table 5). The result also revealed that the mean mycelial growth diameter of fungal isolates on solid medium was maximum at 25°C for all the fungal isolates with the exception of AUA1 which was 30°C (Table 5). Accordingly, the highest mean radial mycelial growth of 90.00±0.00 mm and 85.50±0.50 mm were recorded by AUV1 at 25°C and 30°C, respectively, but mycelial diameter of 63.00±1.00 mm and 54.50±2.50 mm were recorded for isolate AUV2, at temperature ranges of 25–30°C on potato dextrose agar medium, respectively (Table 5). Temperatures below 25°C and above 30°C reduced the growth of fungal isolates to some extent. However, in the present study no growth was observed at 40°C for all the fungal isolates (Table 5).

Table 5. The effect of different temperature on mycelial growth of the fungal isolates after eight days of incubation at 25°C.

Fungal isolates	Mean mycelial growth diameter in mm (Mean±SD)				
	20°C	**25°C**	**30°C**	**35°C**	**40°C**
AUF1	31.67±1.53b	50.50±3.50c	47.50±0.50c	28.00±2.00b	NG
AUA1	27.67±2.52b	37.50±2.50d	39.50±0.50d	26.33±2.08b	NG
AUA2	28.66±3.06b	49.00±3.00c	33.50±3.50e	28.33±7.64b	NG
AUV1	32.66±3.06b	90.00±0.00a	85.50±0.50°	27.00±1.00b	NG
AUV2	50.33±5.51a	63.00±1.00b	54.50±2.50b	48.67±2.08a	NG
Mean±SD	34.20±9.03b	58.00±18.66e	52.90±20.38b	31.67±9.39c	NG

NG= no growth

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test.

The effect of different pH on mycelial growth of fungal isolates

All the fungal isolates were grown between the ranges of 3.0–8.0 pH values tested (Table 6). However, the dry mycelial weight was different among the fungal isolates and different pH levels ($p<0.05$). The maximum mean dry mycelial weight was measured at pH 6.0 by AUV2 (697.6±1.6 mg) followed by pH 5.0 (695.4±2.8 mg) for the same isolate. Similarly, at pH 6.0 maximum biomass (693.9±3.3 mg) was measured followed by 693.2±1.2 mg and 624.5±4.0 mg for AUA1, AUV1 and AUF1, respectively which are not significantly ($p<0.05$) different. At pH 7.0, maximum (356.4±1.1 mg) dry mycelial weight was also measured for AUA2 at pH 5.0 (Table 6).

Table 6. The effect of different pH on the dry mycelial weight of fungal isolates after fifteen days of incubation at 25°C.

	Mean dry mycelial weight in mg of fungal isolates (Mean±SD)				
pH	**AUF1**	**AUA1**	**AUA2**	**AUV1**	**AUV2**
3.0	236.3±4.1c	550.1±3.6a	204.6±3.6d	486.2±4.1b	104.6±2.4e
4.0	494.1±3.4c	574.9±4.3a	227.3±3.0d	530.1±4.9b	167.4±2.4e
5.0	585.7±5.4b	682.5±2.1a	247.2±3.5c	693.4±4.9a	695.4±2.8a
6.0	624.5±4.0b	693.9±3.3a	287.8±2.6c	693.2±1.2a	697.6±1.6a
7.0	471.2±2.6b	592.7±2.0a	356.4±1.1d	372.3±1.5c	294.6±1.8e
8.0	417.1±1.3b	521.0±1.1a	272.6±2.9e	314.1±1.4c	281.5±0.9d
Mean±SD	471.5±37.9c	602.5±19.4a	266.0±14.7e	514.9±43.5b	373.5±71.5d

Each value is an average of two replicates ± standard deviation. Means followed by the same letters within a row are not significantly ($p<0.05$) different according to Duncan's multiple range test.

The effect of carbon sources on the mycelial growth of fungal isolates

The growth of fungal isolates varied ($p<0.05$) among fungal isolates on different carbon sources (Table 7). All the carbon sources supported the growth of fungal isolates even though their growth was different on different carbon sources (Table 7). An isolate (AUF1) grew on dextrose with significantly maximum mycelial growth (76.33±1.84 mm) followed by media amended with maltose (74.86±4.44 mm), D-fructose (64.36±2.20 mm) and sucrose (63.93±1.70 mm). An isolate (AUA1) grew on maltose

with the highest mean colony growth (54.26±1.35 mm) followed by sucrose (53.13±2.19 mm), D-fructose (49.00±1.80 mm) and dextrose (48.16±2.24 mm). The highest mean mycelial growth was recorded on maltose (77.83±1.77 mm) followed by sucrose (74.43±1.60 mm), D-fructose (66.16±1.55 mm) and dextrose (63.13±1.70 mm) in case of AUA2, while AUV1 exhibited maximum growth on dextrose (90.00±0.00 mm) followed by sucrose (89.70±0.51 mm), maltose (89.66±0.57 mm) and D-fructose (80.33±0.77 mm). The maximum mycelial growth was also observed on maltose (81.96±1.90 mm), sucrose (78.13±1.26 mm), D-fructose (77.20±1.55 mm) and dextrose (64.33±1.45 mm) by AUV2 (Table 7).

Table 7. The effect of four different carbon sources on the mycelial growth of fungal isolates after 10 days of incubation at 25°C.

Fungal isolates	**Mycelial growth diameter in mm (Mean±SD)**			
	Dextrose	**Maltose**	**D-fructose**	**Sucrose**
AUF1	76.33±1.84b	74.86±4.44d	64.36±2.20c	63.93±1.70e
AUA1	48.16±2.24d	54.26±1.35e	49.00±1.80d	53.13±2.19f
AUA2	63.13±1.70c	77.83±1.77c	66.16±1.55c	74.43±1.60c
AUV1	90.00±0.00a	89.66±0.57a	80.33±0.77a	89.70±0.51a
AUV2	64.33±1.45c	81.96±1.90b	77.20±1.55a	78.13±1.26b
Mean±SD	68.10±14.50e	73.68±12.86d	69.75±12.26b	71.87±12.97d

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test.

The effect of nitrogen sources on the mycelial growth of fungal isolates

The nitrogen requirement for fungal isolates was studied by using different nitrogen sources and the results are indicated in Table 8. The maximum mycelial growth of the isolate (AUF1) was observed in potassium nitrate (89.33±1.15 mm) followed by urea (63.00±3.00 mm), ammonium chloride (62.66±2.51 mm) and ammonium nitrate (54.33±1.52 mm) (Table 8). Potassium nitrate was found to be a good source of nitrogen for AUF1 (89.33±1.15 mm), AUV1 (88.33±1.15 mm), AUV2 (86.00±2.64 mm) and AUA1 (43.33±3.05 mm). Richard's agar medium amended with ammonium nitrate supported the maximum growth of 72.66±2.08 mm by AUV1. Compared to the other three nitrogen sources, potassium nitrate was the best nitrogen source utilized with mean mycelial growth diameter of 72.33±20.17 mm and urea was the least utilized (41.87±16.62 mm) by all the fungal isolates (Table 8).

Table 8. The effect of four different nitrogen sources on the mycelial growth of fungal isolates after ten days of incubation at 25°C.

Fungal isolates	**Mycelial growth diameter in mm (Mean±SD)**			
	NH_4Cl	**NH_4NO_3**	**KNO_3**	**$[CO(NH_2)_2]$**
AUF1	62.66±2.51b	54.33±1.52b	89.33±1.15a	63.00±3.00a
AUA1	42.66±3.05d	36.33±2.08c	43.33±3.05d	23.00±2.64d
AUA2	26.00±1.00e	69.00±1.00b	54.66±1.52c	39.66±2.08c
AUV1	73.33±2.08a	72.66±2.08a	88.33±1.15a	26.66±3.05d
AUV2	61.33±1.52b	55.33±2.08b	86.00±2.64a	57.00±2.00b
Mean±SD	53.20±17.48c	57.53±13.38b	72.33±20.17b	41.87±16.62c

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test.

DISCUSSION

In the present study, five pathogenic fungal isolates were isolated from infected yam and characterized on the basis of cultural, morphological and eco-physiological characters. The fungal pathogens were identified as *Fusarium* spp. (AUF1), *Alternaria* sp. (AUA1 and AUA2) and *Verticillium* sp. (AUV1 and AUV2). Similarly, Mathur and Kongsdal (2003) have identified *Fusarium* sp., *Alternaria* sp., *Verticillium* sp. and other fungal pathogens of crops from various seed sources.

The data showed that AUA1 and AUA2 produced light brown and muriform shape of conidia measuring about 30.5–40.47 x 10.9–17.37 µm and 27.3–42.5 x 11.6–16.9 µm, respectively. Similarly, Ramjegathesh and Ebenezer (2012) recorded length and width of conidia (30.99–42.47 x 11.9–17.37 µm) in *Alternaria alternata* isolates. Likewise, AUF1 has banana-shaped conidia with foot cells in sporodochia and has three septate with conidial measurement of 19.0–26.2 x 3.2–5.8 µm. Similarly, Sunita (1999) has reported that such characters belong to *Fusarium oxysporum*. Smith (2007) has also reported that the microconidia of *Fusarium oxysporum* were typically 25–35 x 3–5 µm wide, dorsi-ventrally curved, sickle-shaped with 3–5 septated structure. The range of conidial measurement of AUV1 and AUV2 were 3.5–11.2 x 1.8–3.8 µm and 2.5–9.0 x 1.25–3.2 µm, respectively with hyaline, ovoid-ellipsoidal shape produced at the apices of phialides. Similarly, these conidial features were reported by Jabnoun-Khiareddine *et al*. (2010) who have recorded that the conidial measurement of *Verticillium tricorpus* was 3.75–6.25 x 2.5 µm, based on the study of morphological variability among the *Verticillium* species.

The confirmation of pathogenicity studies indicated that the symptoms observed under artificial conditions were similar to the natural symptoms noticed on yam plants. Adeniji (1970) has reported that fungi are associated with storage decay of Nigerian yams. The inoculation and re-isolation

experiments also confirmed that all the five fungal isolates were associated with yam diseases and these fungi were capable to induce typical disease symptoms. Also, Noon and Calhoun (1979) have reported that the pathogenicity of *Fusarium* spp. isolates revealed that they were able to rot the healthy yam tubers into which they were inoculated. The front side of re-isolated culture imparted pink, dark gray, gray, whitish and white colours for AUF1, AUA1, AUA2, AUV1 and AUV2 on PDA medium, respectively, which was the same as the original culture.

Optimized growth conditions (media, temperature, and pH) of the pathogen are routinely used as a starting point for *in vitro* and *in vivo* evaluation of the efficacy of different fungicides against the fungal isolates. In the present study, the fungal isolates exhibited variations of growth on different culture media tested. Among the four culture media evaluated, maximum radial growth of all the fungal isolates were achieved on PDA medium followed by MEA and CDA. Thus, PDA medium was the best preferred medium for the growth of fungal isolates compared to the three media. Similarly, Kulkarni (2006) has reported that among the solid culture media evaluated, maximum radial growth of *Fusarium oxysporum* was observed on PDA followed by Richards's agar medium. Ashraf *et al*. (2012) have reported that isolates of *Verticillium dahliae* causing wilt of potato showed the maximum mean mycelial growth percentage on MEA followed by PDA culture medium. These findings agree well with the results of the present study in which *Verticillium* spp. attained maximum mycelial growth on PDA followed by MEA culture media.

It is possible to conclude that from the present result, each fungal isolate had their temperature range for growth and sporulation. The maximum mycelial growth of fungal isolates was obtained at 25°C for all the fungal isolates with the exception of AUA1 which showed maximum mycelial growth (39.50±0.50 mm) at 30°C. Thus, optimum temperature of fungal isolates ranged between 25–30°C. Similarly, Khan *et al*. (2011) have indicated *Fusarium* spp. grew best between 25°C and 30°C. Ramteke and Kamble (2011) have also reported that *Fusarium solani* grew at temperatures ranging from 10 to 35ºC, with optimum growth at 25ºC and no growth was observed at 5ºC and 40ºC which was in agreement with the present finding that all the fungal isolates did not grow at 40ºC. According to Hubballi *et al*. (2010), the results of the experiment indicated that the mycelial growth of *Alternaria alternata* was maximum at temperature range of 25–30°C. Ashraf *et al*. (2012) have reported on the mycelial growth of *Verticillium dahliae* isolated from potato wilt at different temperature ranging from 18–

30ºC and found that the temperature value of 26ºC was the best suited for the mycelial growth of *Verticillium dahliae* which is consistent with the present finding.

The present result also indicated maximum dry mycelial weight of AUF1 with an optimum pH range of 5.0 to 6.0. Similarly, Gangadhara *et al*. (2004) have reported that the most suitable pH value for growth of *Fusarium oxysporum* was 5.0 and 6.0. However, Khan *et al*. (2011) have shown that optimum pH for growth of *Fusarium oxysporum* ranged from 6.5 to 7.0. With regard to AUA1, the dry mycelial weight was maximum (693.9±3.3 mg) at pH 6.0. Hubballi *et al*. (2010) have also reported that the highest mycelial growth of *Alternaria alternata* under *in vitro* condition was obtained at the maximum pH range of 6.0–6.5. From the result, AUA2 grew at pH 7.0 which accumulated maximum dry mycelial weight of 356.4±1.1 mg followed by pH 6.0 (287.8±2.6 mg). Among the pH values tested, the maximum biomass was measured at pH 7.0 (693.4±4.9 mg) for AUV1 and at pH 6.0 (697.6±1.6 mg) for AUV2. Thus, the pH values below five and above seven were inhibitory to the growth of fungal isolates. Similarly, Bhat *et al*. (2003) have reported that the growth of *Verticillium* spp. occurred over a wide range of pH 4.5–7.0 with the optimum at pH 5.0. Ashraf *et al*. (2012) also reported maximum growth of *Verticillium dahliae* that causes wilt in potato plants at pH 5.0 after seven days of incubation.

Knowing the nutritional requirements and factors influencing the growth of pathogen is a precondition for any study leading to the understanding of host-pathogen relationships. Carbon and nitrogen are involved in mechanisms like host-pathogen interaction and self-defense mechanisms since they are the main components of carbohydrates, proteins, lipids and nucleic acids. In the present study, four different carbon sources were tested in solid Richards' agar medium to understand their effect on the growth of the fungal isolates. From the carbon sources studied, maximum growth was recorded by AUV1, with mycelial growth of 90.00±0.00 mm on media amended with dextrose followed by sucrose (89.70±0.51 mm) and maltose (89.66±0.57 mm). Ramjegathesh and Ebenezer (2012) have reported that among the various carbon compounds tested, maltose supported the maximum mycelial growth (88.2 mm) followed by glucose (86.7 mm) and sucrose (82.7 mm) for *Alternaria alternata* which is in agreement with the present study with maximum mycelial growth of 54.26±1.35 mm for AUA1 and 77.83±1.77 mm for AUA2 on the media amended with maltose. In this study, the maximum growth (76.33±1.84 mm) was recorded for AUF1 on the media amended with dextrose. Similarly, Li (2011) reported that

maximum growth (59.6 mm) was supported by glucose in the case of *Fusarium* sp.

With regard to the nitrogen sources, nitrogen was found to have a profound effect on growth and metabolism of fungal isolates because it is an important element for protein synthesis. In the present study, among the nitrogen sources tested, potassium nitrate supported the maximum growth (69.00±1.00 mm) for all fungal isolates, except AUA2 on media amended with ammonium nitrate. Similarly, Ramjegathesh and Ebenezer (2012) have reported that among the various nitrogen sources tested, potassium nitrate supported the maximum growth (90.0 mm) followed by sodium nitrate (73.2 mm) for *Alternaria alternata*. Dandge (2012) has also reported that potassium nitrate was an effective source for the growth of *Fusarium* spp. The present study indicated that all the fungal isolates had their optimum temperature and pH ranges for their growth and reproduction.

The present study implies that media optimization is important to understand the cultural characteristics of the pathogens, so that it can be used to prepare the medium for the fungal pathogens. All the fungal pathogens had their preferred optimum temperature and pH ranges for growth and reproduction. The study had limitations in laboratory facilities which resulted in the identification of fungal pathogens using conventional techniques. It is very important to have identification using molecular techniques in addition to the conventional identification techniques. There is a need to undertake an intensive research on diseases of yam crops in all yam growing areas of Ethiopia and quantification of yield loss by pathogens.

CONCLUSION

Different cultural and morphological characteristics indicated that the fungal isolates belonged to the genus *Fusarium* (AUF1), *Alternaria* (AUA1 and AUA2) and *Verticillium* (AUV1 and AUV2). An *in vitro* pathogenicity test confirmed that the fungal isolates were pathogens of yam. The present study indicated that mycelial growths of fungal isolates were influenced by culture media, temperature, pH, carbon and nitrogen sources. All the fungal isolates grew best on PDA medium followed by MEA culture medium. The optimum temperature for best mycelial growth of all fungal isolates ranged between 25–30ºC. A pH value below 5.0 and above 7.0 was inhibitory to the growth of fungal isolates. Among the carbon sources tested, AUF1 and AUV1 grew profusely on dextrose and AUA1, AUV2 and AUA2 on maltose. From the nitrogen sources tested, potassium nitrate was the best

source utilized by all fungal isolates, except AUA2, which grew best on ammonium nitrate.

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