ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY

ISOLATION, CHARACTERIZATION OF FUNGAL PATHOGENS OF YAM FROM SEKA CHOKERSA WOREDA, JIMMA ZONE AND ITS CONTROLS USING *TRICHODERMA* SPP AND FUNGICIDES

BY
BIKILA WEDAJO

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN APPLIED MICROBIOLOGY

JANUARY, 2014

ADDIS ABABA, ETHIOPIA

ISOLATION, CHARACTERIZATION OF FUNGAL PATHOGENS OF YAM FROM SEKA CHOKERSA WOREDA, JIMMA ZONE AND ITS CONTROLS USING *TRICHODERMA* SPP AND FUNGICIDES

BY

BIKILA WEDAJO

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN APPLIED MICROBIOLOGY

ADVISOR

Dr. TESFAYE ALEMU

JANUARY, 2013

ADDIS ABABA, ETHIOPIA

DECLARATION

I, the undersigned declared that the work which is being presented in the thesis entitled, "Isolation, characterization of fungal pathogens of yam from Seka Chokersa Woreda, Jimma Zone and its controls using *Trichoderma* spp and fungicides" in partial fulfillment of the requirements for the award of the degree of Masters of Science in Applied Microbiology, Department Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University; is an authentic record of my own work under the supervision of Dr. Tesfaye Alemu. All materials used in this thesis have been dually acknowledged.

Approved by Examining Board:

Dr. Fassil Aseffa (Examiner): _____

Dr. Diriba Muleta (Examiner): _____

Dr. Tesfaye Alemu (Advisor): _____

ACKNOWLEDGMENT

I would like express my deepest gratitude to my advisor, Dr. Tesfaye Alemu, for his supervision, advice and guidance from proposal writing to the completion of this thesis work. I really appreciate his friendly advice and genuine guidance, close supervision at each and every step, critical comments of the manuscript of the thesis and immediate feedback with valuable comments.

Apart from personal efforts and steadfastness to work, constant inspiration and encouragement given by a number of individuals served as the driving force that enabled me to submit this thesis in the present form. I would like to pass my special thanks to Mycology laboratory technicians, Zenebech Aytenew and Nigat Mekonnen, for their help, support and making available laboratory materials. My sincere thanks to all my colleagues who have directly or indirectly helped me has been instrumental in the smooth completion of this work.

I would like to extend my sincere thanks to Gutu Adugna for his help during statistical analysis of data, Zerihun Belay for his technical support and Ayele Gucho for his pleasant help and material support until the completion of my study. Also, I would like to extend my deepest gratitude to all members of my families and relatives for their unlimited help and prayer.

Finally, I would like to acknowledge Arba Minch University and Addis Ababa University, especially School of Graduate Study, Department of Microbial, Cellular and Molecular Biology for all round assistance and allowing me to carry out the research in the laboratory, and for financial support during my research work.

Above all, I would like to thanks to Almighty God, who made me all my way comfortable and do everything to me previously through and in the future throughout my life and without of which His help my work would not be completed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS

BCAs= Biological Control Agents

CDA= Czapeck Dox‟s agar

IITA= International Institute of Tropical Agriculture

MEA= Malt extract agar

NCA= North and Central America

NG= no growth

PDA= Potato Dextrose Agar

PDB= Potato Dextrose Broth

SDA= Sabouraud‟s Dextrose Agar

SNA= Synthetic Nutrient-Poor Agar

WP= wettable powder

ABSTRACT

In the present study, five different fungal isolates (AUF1, AUA1, AUA2, AUV1 and AUV2) were isolated from infected yam leaves and tubers grown in Seka Chokersa woreda, Jimma Zone, Ethiopia based on cultural and morphological. The ranges of conidia (length by width) of AUF1, AUA1, AUA2, AUV1 and AUV2 were 19.00-26.20×3.20-5.80 µm, 30.50-40.47×10.90-17.37 µm, 27.30-42.50×11.60-16.90 µm, 3.50-11.20×1.80-3.80 µm and 2.50-9.00×1.25-3.20 µm, respectively. The pathogenicity test showed that the most virulent isolates were AUF1, AUA1 and AUV1 with 100% tissues infection. Growth characteristics of the isolates showed that isolate AUF1 and AUV1were best grown on PDA medium with mycelial diameter of 90 mm followed by AUA1, AUV2 and AUA2, respectively. Similarly, the isolates grew best at 25^{o}C, with the exception of AUA1 that displayed maximum growth at 30 ^{o}C. From the pH levels studied, maximum dry mycelial weight was attained from AUV2 (697.6±1.6 mg), AUA1 (693.9±3.3 mg) and AUF1 (624.5±4.0 mg), at pH 6.0; whereas, dry mycelial weight of 693.4±4.9 mg and 356.4±1.1 mg at pH 5.0 and 7.0 measured from AUV1 and AUA2, respectively. Dextrose was the most utilized carbon source by AUF1 and AUV1; while maltose was the best carbon source for AUA1, AUA2 and AUV2. Potassium nitrate was the best nitrogen source for all fungal isolates with the exception of AUA2 that utilized ammonium nitrate best. In vitro evaluation of dual culture test with Trichoderma spp against the fungal pathogens revealed that both of them showed effective with 66.75 to 82.59% of mycelial growth inhibition. Similarly, curzate (43.93% WP) and sancozeb (80% WP) were effective at high concentrations of (400-1000 ppm), where sancozeb was more effective than curzate in terms of percent growth inhibition. From the study of combined treatment of the pathogens with fungicides and Trichoderma isolates, the highest percent of inhibition of mycelial growth of 85.6%, 79.7%, 87.5%, 89.3% and 80.2% were detected when AUT2 was combined with sancozeb against AUF1, AUA1, AUA2, AUV1 and AUV2, respectively. So, combination of Trichoderma spp (AUT1 and AUT2) with chemical fungicides (curzate and sancozeb) at lower concentration offers a promising control of fungal pathogens.

Key words: Biocontrol agents, *Dioscorea* spp, Fungal isolates, *In vitro*

1. INTRODUCTION

Root and tubers crops comprise crop covering several genera. They are staple food crops, being the source of daily carbohydrate intake for the large population of the world. The tuber refers to any growing plant store edible materials in subterranean root, corm or tuber from which yam is a member of this important class of food (Oke, 1990). Yams are plants belonging to the family *Dioscoreaceae,* genus *Dioscorea* and are cultivated for their edible tubers, which in some species can grow up to about 2.4 m long and with weight up to 45 kg (Kay, 1987). It is a versatile vegetable that can be roasted, fried, grilled, boiled, baked, smoked and when grated (reduced to small shreds by rubbing) it is processed into a dessert recipe (Huxley, 1992). *Dioscorea* spp has also medicinal properties as their tubers contain diosgenin, a biochemical precursor in the synthetic production of progesterone and other corticosteroids (Albrecht and McCarthy, 2006).

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 (Okigbo and Ogbonnaya, 2006). In West African yam zone, it account for over 90% of the 4.59 million hectares of yam cultivation worldwide which is estimated at about 20-25million tons per year (Izekor and Olumese, 2010). Ethiopia and Sudan are major yam producers in East Africa (Musa *et al*., 2011). The yam plants are mostly distributed in south and south west Ethiopia around Metekel, Sidama, Wolayta, Gambella, Maji, Jimma and North Omo (Umeta, 2005). Ethiopia has a world share of 0.6% yam production (tons) and 0.9% area harvested (hectare) in the world (FAO, 2011). Nigeria is the main producer of yam in the world with about 71% of the world output followed by Cote d'Ivoire (8.1%), Benin (4.3%) and Ghana (3.5%) (Odior and Oyawale, 2012). It accounts for over 50% of the total daily carbohydrate consumption of the average Nigerian population (Suleiman, 2010).

There are several limiting factors for the production, processing and quality of yam in the world. Yam plants are susceptible to infection by fungi, bacteria and viruses at all stages of growth and also during storage of tubers. Tuber rot is a major factor limiting the post-harvest life of yams and losses can be very high which is estimated at about 26% in the world (Amusa *et al*., 2003). Rot is the process of decomposition or decaying of tubers by the action of fungi and bacteria. Most rots of yam tubers in the storage are caused by pathogenic fungi such as *Aspergillus flavus,*

Aspergillus niger, Botryodiplodia theobromae, Fusarium oxysporum, Fusarium solani, Penicillium chrysogenum, Rhizoctonia spp, *Penicillium oxalicum* and *Rhizopus nodosus* (Okigbo and Ikediugwu, 2002; Okigbo and Emoghene, 2004).

The quality of yam tubers are affected by rots, which makes them unpleasant to consumers. In Nigeria, over 60% of white yam varieties get rotten when stored for less than six months (Adesiyan and Odihirin, 1975). Similar situations or worse can be found in Ghana. It has been observed that from farmers‟ fields and discussions reveal that some farmers lose as high as 70% of their stored yam to rot causing fungal pathogens (Aidoo, 2007).

Apart from tuber rots, yam production and marketing are threatened by a variety of fungal pathogens in the fields. 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 and at all stages of crop development (Akem, 1999). The diseases 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).

More of the fungal pathogens of yam were known in many countries, but in Ethiopia there is no information concerning pathogens of yam. So, understanding of the role of environmental conditions and its effect on the infection and survival of pathogen is necessary to develop cultural, biological and chemical disease management practices. The hypothesis set for research is that media type, temperature, pH, carbon and nitrogen sources will influence the growth of fungal pathogens and controlling these fungal pathogens through biocontrols and fungicides will be effective.

Similarly, in Ethiopia fungal diseases are the most important factors that contribute to the reduction of yam production. Leaf spot of yam diseases caused by *Alternaria* species infect yam crops in the major yam growing areas of Ethiopia (Van Bruggen, 1984). Therefore, this study was designed to isolate, characterize, and conduct pathogenicity test and *in vitro* evaluation of biological control agents and fungicides against fungal diseases of yam.

2. OBJECTIVES OF THE STUDY

2.1. General objective

The general objective of the current study was:

- To isolate and characterize fungal pathogens of yam *in vitro* and to evaluate the effect of *Trichoderma* isolates and fungicides against these infectious agents

2.2. Specific objectives

The specific objectives of this project were:

- To isolate, identify and characterize fungal pathogens of yam tubers and leaves from yam growing areas.
- To optimize the cultural conditions (culture media, temperature, pH) of the isolates under laboratory condition.
- To study the pathogenicity of the isolates on the host and re-isolation of fungal pathogens.
- *In vitro* evaluation of *Trichoderma* isolates and fungicides against the isolates.
- To screen *Trichoderma* spp tolerance to fungicides (curzate and sancozeb).

3. LITERATURE REVIEW

3.1. Description of yam plant

Yam is the common name for members of the genus *Dioscorea* (family *Dioscoreaceae).* The word „yam‟ comes from Portuguese *inhame* or Spanish *ńame*, which both ultimately derives from the Wolof word *nyame*, meaning "to sample" or "taste", in other African languages it can also mean "to eat" such as yamyam and nyama in Hausa (Mignouna and Dansi, 2003). Also yam is the common name for some species in the genus and they are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America and Oceania (Adetuyi *et al*., 2010). They are used in a fashion similar to potatoes and sweet potatoes (Brand-Miller *et al*., 2003). Yam products generally have a lower glycemic index than potato products (Kay, 1987), which means that they will provide a more sustained form of energy, and give better protection against obesity and diabetes (Walsh, 2003).

The inconspicuous white or greenish-yellow flowers of yams, arranged in spikes or racemes, have a six-parted calyx (outer flower whorl), a six-lobed corolla (inner floral whorl), six stamens, and a solitary pistil. The fruit of the yam consists of a membranaceous, three-winged capsule (Kay, 1987). Yam shoots are climbing and vine-like thus usually requiring support from either neighboring plants or stakes on which they twine clockwise or anticlockwise depending on the species. Shoots can be round, winged or ridged, some bearing spines at the base. Leaves are opposite, heart-shaped, ovate to oblong, with a deeply cordate base. They are 3-6 inches long and have 7-9 veins that begin at the leaf base. Yam fruits are dry capsules, 1-2 cm long and usually broader than long. Seeds are flattened, hard and encircled by a wing. The tuber is roughly cylindrical in shape, the skin is smooth and brown and the flesh usually white and firm (Johnson, 2003).

The yam crop has a rough skin which is difficult to peel, but which softens after heating. The skins vary in color from dark brown to light pink. The majority of the vegetable is composed of a much softer substance known as the "meat". This substance ranges in color from white or yellow to purple or pink in mature yams. Some varieties of these tubers can be stored up to six months without refrigeration, which makes them a valuable resource for the yearly period of food scarcity at the beginning of the wet season (Huxley, 1992).

3.2. Different types of yam cultivars

Yam is a multi-species, polyploid and vegetatively propagated tuber crop that is cultivated widely in the tropics and subtropics. The number and shape of yam tubers vary largely between species (FAO, 2002). The tubers can range in size from that of a small potato to behemoths over 71/2 feet long and 120 pounds (Huxley, 1992). Depending on the variety, a yam's flesh may be of various shades of off-white, yellow, purple, or pink, and the skin from off-white to dark brown. The texture of this vegetable can range from moist and tender to coarse, dry, and mealy. Yams can be found in most Latin American markets, often in chunks, sold by weight (IITA, 2004).

There are more than 600 species of yam. Some of the cultivated species are *Dioscorea rotundata* (White yam), *Dioscorea dumetorum* (Bitter yam), *Dioscorea cayensis* (Yellow yam), *Dioscorea alata* (Water yam), *Dioscorea esculenta* (Chinese yam) and *Dioscorea bulbifera* (Aerial yam) (Kay, 1987). But the edible yams are derived mainly from the most economically important species, which is; White yam (*Dioscorea rotundata* Poir). Originated in Africa and is the most widely grown and preferred yam species. A large number of white yam cultivars exist with differences in their production and post-harvest characteristics. It has a long shelf-life which does not affect its cooking and organoleptic qualities and can be available all year round (Thomas, 2012).

Yellow yam (*Dioscorea cayenensis* Lam.) which derives its common name from its yellow flesh is also native to West Africa and very similar to the white yam in appearance. Its yellow flesh is caused by the presence of carotenoids. Because of the tuber skin is firm and less extensively grooved, the yellow yam has a longer period of vegetation and a shorter dormancy than white yam (Mignouna and Dansi, 2003). Water yam (*Dioscorea alata* L.) originated from South East Asia, it is the species most widely spread throughout the world and in Africa is second only to white yam in popularity. According to Lebot *et al*. (2005), it is the most widely distributed species in the humid and semi-humid tropics. The tuber shape is generally cylindrical, but can be extremely variable. Tuber flesh is white and watery in texture. It is an important food in Africa, the Caribbean, and especially Melanesia where it has considerable social and cultural importance (Lebot *et al*., 2005). Bitter yam (*Dioscorea dumetorum*) is also called trifoliate yam because of its leaves. Originates in Africa where wild cultivars also exist. One marked characteristic of the

bitter yam is the bitter flavor of its tubers. Another undesired characteristic is that the flesh hardens if not cooked soon after harvest. Some wild cultivars such as *Dioscorea cayenensis*, *Dioscorea hispida* and *Dioscorea bulbifera* are highly poisonous (Mignouna and Dansi, 2003).

Depending on the species, yam grows for six to ten months and is dormant for two to four months. These two phases usually corresponds to the wet season and the dry season. For maximum yield the yam requires an annual rainfall of over 1,500 mm distributed uniformly throughout the growing season. White, yellow and water yams normally produce a single large tuber, often weighing 5-10 kg annually. *Dioscorea rotundata* and *Dioscorea cayenensis* are the most popular and economically important yams in West and Central Africa where they are indigenous, while *Dioscorea alata* is the most widely distributed species globally (Mignouna and Dansi, 2003).

3.3. Nutritional composition of yam cultivars

Yam is an important source of carbohydrate for many people of the Subsaharan Region, especially in the yam zone of West Africa (Akissoe *et al*., 2003). Babaleye (2003) has reported that yam contributes more than 200 dietary calories per capita daily for more than 150 million people in West Africa and serves as an important source of income to the people. Yam has some inherent characteristics, which make it attractive, first, it is rich in carbohydrate especially starch consequently has a multiplicity of end use. Secondly, it is available all year round making it preferable to other seasonal crops (FAO, 1987).

Yam shows nutritional superiority over other tropical root and tuber crops. Yams are the most nourishing plants in the diet of many inhabitants of inter-tropical region. The nutritional value of yam confirms its importance to mankind. It is a major source of carbohydrate and is one of the cheaper sources of carbohydrate to man (Izekor and Olumese, 2010).Yam contains protein and substantial amount of vitamins (Thiamine, Riboflavin and Ascorbic acid) and some other minerals like calcium, phosphorus and iron than any other common tuber crop. By dry weight yam is composed of 80% starch, 7% protein, 7% minerals, 3% fibre and 1.7% lipids; 100 g of the yam give 385kcal energy (Olaoye and Oyewole, 2012). Yam has other uses other than food. The yam tuber is a good source of energy mainly from their carbohydrate contents since it is low in fat and protein. Yam tuber is said to contain some pharmacologically active substances such as

dioscorine, saponin and sapogenin. Dioscorine which is the major alkaloid in yam is medicinally a heart stimulant (Eka, 1985). Also it has been reported that yam is a good source of industrial starch whose quality varies with the species (Shehu *et al*., 2010).

Yam is far from being a balanced food and deficiency diseases are prevalent within the poorer population of yam growing areas, but yam is greatly superior to cassava, sweet potato and taro with regard to protein and vitamin C and its replacement by the more easily growing cassava may be a factor for the spread of deficiency diseases in many tropical regions. *Dioscorea alata* cultivars possess a higher content of protein, vitamin C and low lipids than *Dioscorea cayenensis*, *Dioscorea escunlenta*, *Dioscorea rotundata* and *Dioscorea trifida* (Muzac-Tucker *et al*., 1993). *Dioscorea dometorum* is a yam species with the highest nutritional qualities, containing high protein and mineral values (Olaoye and Oyewole, 2012). Post-harvest losses are large in *Dioscorea dometorum*, because if tubers are not cooked within a few days after harvest they become hard and inconsumable (Afoakwa and Sefa-Dede, 2002b).

Yams have high moisture, dry matter, starch, potassium and low vitamin A contents. It also contains high potassium and manganese and low in saturated fat, sodium and cholesterol (Walsh, 2003). The high potassium and low sodium content of yam produces good potassium-sodium balance in the human body and so protect against osteoporosis and heart disease (Walsh, 2003). Slow break down of carbohydrates and gradually release of glucose into the blood stream means that they will provide more sustained form of energy, and give better protection against obesity and diabetes (Brand-Miller *et al*., 2003). Yams contain about 5-10 mg per 100 g vitamin C, and the limiting essential amino acids are isoleucine and those containing sulphur. Some species contain alkaloids such as dioscorine ($C_{13}H_{19}O_2N$) and steroid derivatives. Table 1 indicates a summary of the nutritional values of yams while Table 2 shows the nutritional values for individual yam species. It should be noted that the method of preparation affects the final nutritional status of yam based foods. These data are useful in designing new product formulations as well as efficient food process operations.

Table1 Range of nutritional values of yam (nutrients in 100 g edible portion)

Nutrient	**Tuber**	**Bulbils**
Calories	71.00-135.00	78.0
Moisture (%)	81.00-65.00	79.4
Protein (g)	1.40-3.50	1.4
Fat (g)	0.40-0.20	0.2
Carbohydrates(g)	16.40-31.80	18.0
Fibre (g)	0.40-10.00	1.2
Ash	0.60-1.70	1.0
Calcium (mg)	12.00-69.00	40.0
Phosphorous (mg)	17.00-61.00	58.0
Iron (mg)	0.70-5.20	2.0
Sodium (mg)	8.00-12.00	-
Potassium (mg)	294.00-397.00	-
b -Carotene eq. (mg)	0.00-10.00	-
Thiamin (mg)	0.01-0.11	-
Riboflavin (mg)	0.01-0.04	-
Niacin (mg)	0.30-0.80	-

Source: (Opara, 1999).

Table 2 Nutrient content of yam species (*Dioscorea* spp.) per 100 g edible tuber portions

Nutrient content	*Dioscorea* spp	*D. alata*	*D. bulbifera*	*D. cayenensis*	*D. esculenta*	*D. rotundata*
Water (ml)	69	65-76	71-(79)‡	80	70-74*	80
Calories	119	135-87	112-(78)	71	112-102	71
Protein (g)	1.9	2.3-1.9	1.5-(1.4)	1.5	3.5-1.5	1.5
Fat (g)	0.2	0.1-0.2	0.1-(0.2)	0.1	0.1-0.2	0.1
Carbohydrate (g)	27.8	31-20	26-(18)	16	25-24	16
Fibre (g)	0.8	1.5-0.6	0.9-(1.2)	0.6	0.5-0.6	0.6
Calcium (mg)	52	28-38	69-(40)	36	62-12	36
Phosphorous (mg)	61	52-28	29-(58)	17	53-35	17
Iron (mg)	0.8	1.6-1.1	(2.0)	5.2	0.8	5.2
Vitamins						
carotene eq. (µg)	10	10-5				
Thiamine (mg)	0.11	0.05-0.10			0.10	
Riboflavin (mg)	0.02	0.03-0.04			0.01	

‡Bulbil or aerial tuber; *two values reported; Source: (Opara, 1999).

3.4. Antinutritional components of yam cultivars

The edible, matured, cultivated yam does not contain any toxic principles. However, bitter principles tend to accumulate in immature tuber tissues of *Dioscorea rotundata* and *Dioscorea cayenensis*. They may be polyphenols or tannin like compounds. Wild forms of *Dioscorea dumetorum* do contain bitter principles, and hence are referred to as bitter yam. The bitter principle has been identified as the alkaloid dihydrodioscorine, while that of the Malayan species, *Dioscorea hispida*, is dioscorine. These are water-soluble alkaloids, which, on ingestion, produce severe and distressing symptoms. Severe cases of alkaloid intoxication may prove fatal. There is no report of alkaloids in cultivated varieties of *Dioscorea dumetorum*. The bitter principles of *Dioscorea bulbifera* include a 3-furanoside norditerpene called diosbulbin. These substances are toxic, causing paralysis. Extracts are sometimes used in fishing to immobilize the fish and thus facilitate capture (Shanthakumari *et al*., 2008). Toxicity may also be due to saponins in the extract. Zulus use this yam as bait for monkeys, and hunters in Malaysia use it to poison tigers (Ogbuagu, 2008). In Indonesia an extract of *Dioscorea bulbifera* is used in the preparation of arrow poison (Sanni *et al*., 2003).

In addition to their very important use as human food and their ceremonial significance, some wild species of yam such as *Dioscorea villosa* are known to contain steroidal saponins and sapogenins which are precursors for cortisone used medicinally for the management of menopausal symptoms and treatment of arthritis and menstrual disorders (Komesaroff *et al*., 2001). Other wild species are cultivated for extraction of diosgenin, a female hormone precursor used in the manufacture of contraceptive pills and sex hormones (Kay, 1987; Ulbricht *et al*., 2003).

3.5. Importance of yam cultivars

Yams are one of the most highly important food products in tropical countries of West Africa and are closely integrated into social, economic, cultural and religious aspects of communities. The ritual ceremony and superstition often surrounding yam and its utilization in West Africa is a strong indication of the antiquity use of this crop. Their high values as a food source, some species of yam have been used as medicine to treat diseases like diabetes mellitus, coronary disorders and in preventing high hypercholesterolemia (an excess of cholesterol in the

bloodstream). Yam starch takes longer time to break down compared with other starchy tubers like potato and sweet potato, which makes it a safer source of carbohydrate for diabetics. It is a good source of manganese, a vital micronutrient (Okigbo and Ogbonnaya, 2006). Some yam species such as *Dioscorea piscatorum* have toxic properties that allow them to be used in the production of insecticides. An insecticide from *Dioscorea piscatorum* is used in controlling insect pests of rice in Malaysia. Extracts from *Dioscorea deltoidea* is used in the production of anti-lice shampoo in India (Coursey, 1967a).

3.6. Yam production and consumption

The major yield limiting factors for tuber and root crops production and quality in West**,** Central and East African countries is diseases of fungal, bacterial, viral and nematodes**.** These diseases infect and affected tuber and root crops at all stages of development. Some of these diseases devastated the leaves, stems, roots and floral parts of the crops there by inhibiting the transport of food and water which finally results in the total death of the tuber and root crops. Its major limitation in the field is the susceptibility of most cultivars to anthracnose disease that exerts a devastating impact on productivity of yam and its quality. Epidemics that commence prior to or during tuber formation can have a tremendous effect on tuber yield (Mathew *et al.,* 2003).

The most dominant production and consumption zone for yams in the world is in West and Central Africa. It is eaten in different forms as fufu/chips, boiled, fried and roasted. The crop serves as a major source of foreign exchange earnings and is used as raw materials for starch industries and pharmaceutical companies (Amanze *et al*., 2011). Most of the world production of yam is from Africa (about 96%) with Nigeria alone accounting for nearly 75% of the total world production. During the period 1975-1990, total yam cultivated area increased by about 38.8% globally, while the total production increased by 45.8%. However, the importance of yam in the economy of the main producing areas appears be declining due partly to competition with other crops like cassava in Nigeria, and taro in the South Pacific (Opara, 1999). Table 3 shows the summary of world production of yam (*Dioscorea* spp).

Table 3 World production of yam (*Dioscorea* spp)

	Area (10^3 ha)	% of World Area	Production (10^3 Mt)	% of World production	Yield (Mt.ha^{-1})	% of World Yield
World	2,110	100	20,198	100	9.6	100
Africa	2,049	97.1	19,539	96.7	9.4	97.9
NCA	22	1.0	243	1.2	11.1	115.6
SA	10	0.5	48	0.2	4.7	0.5
Asia	15	0.7	168	0.8	11.4	118.8
Oceania	15	0.7	200	1.0	13.5	140.6
Leading countries						
1. Nigeria	1,350	64.0	15,000	74.3	11.1	115.6
2. Côte d'Ivoire	200	9.5	1,700	8.4	8.5	88.5
3. Ghana	160	7.6	800	4.0	5.0	52.1
4. Togo	100	4.7	750	3.7	7.5	78.1
5. Benin	59	2.8	610	3.0	10.3	107.3

10^3 ha= area on thousand hectare, 10^3 Mt=production in thousand metric tons, Mt.ha^{-1}=yield in metric tons per hectare, NCA=North and Central America, SA=South America

Source: Adopted from (FAO, 1975)

3.7. Yam processing

Primary products of yams are mainly grown for direct human consumption and are marketed as fresh produce in all the growing regions. Common methods of preparation include boiling, baking or frying. Boiled and baked yam can be eaten with vegetable sauce or palm oil. Boiled yam can also be pounded or mashed in mortar and eaten as “fufu” or “utara”. Commercially food processing equipment for boiling and mashing of yam into fufu at the press of a button are now available in the market. Secondary and derived products of yam tubers are also processed into several food products such as the yam flour (Opara, 2001). The production process of instant pounded yam consists of simple operations (Figure 1) and they are yam selection, peeling, washing, cutting, flaking, blanching, drying, milling and packaging (Olaoye and Oyewole, 2012)

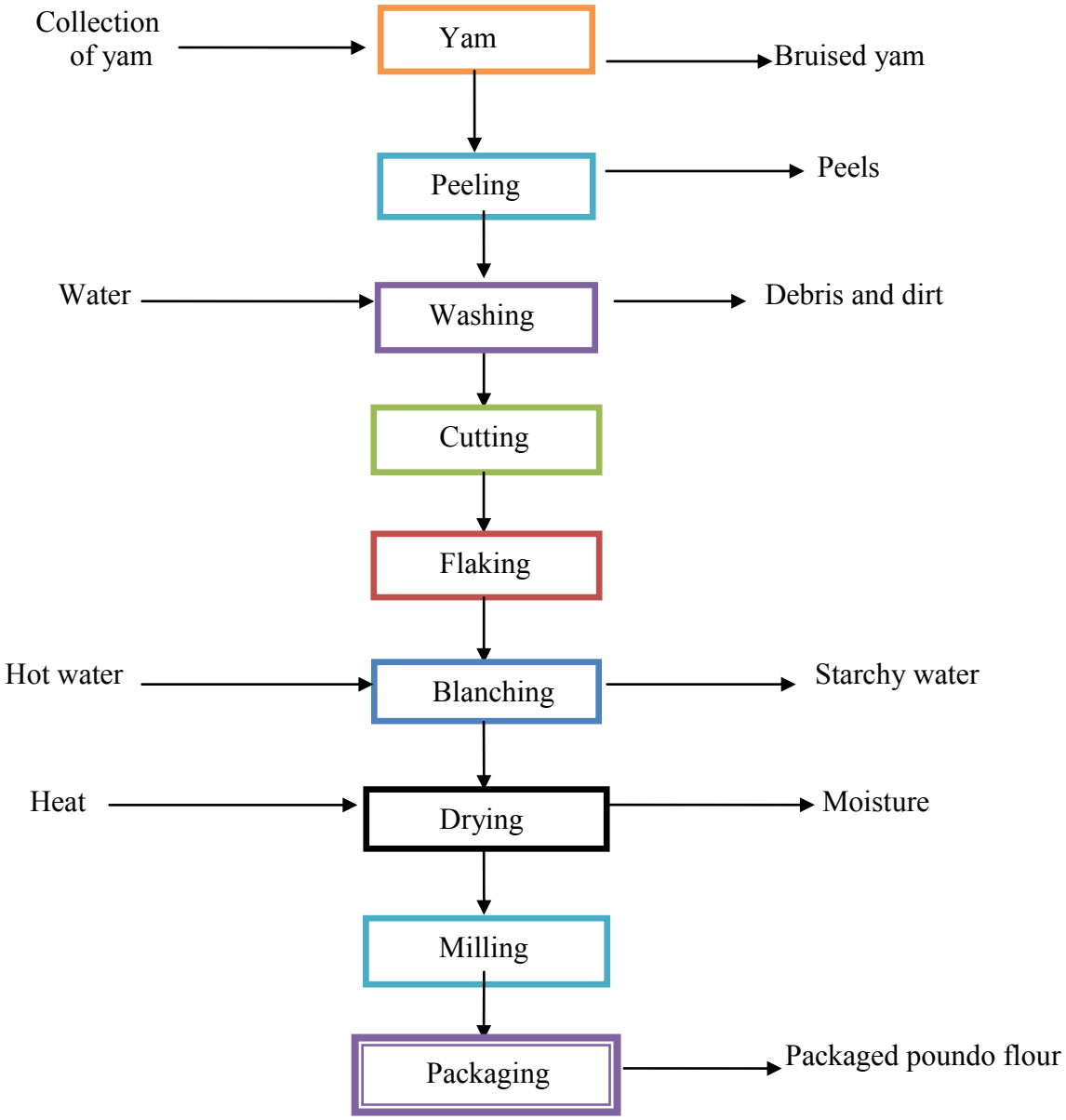


Figure 1 Flow diagram for poundo yam flour production process (Olaoye and Oyewole, 2012)

3.8. Diseases of yam

Post-harvest losses account for a reduction of about 26% in world yam production (Amusa *et al*., 2003). The annual post-harvest yam loss in West Africa estimated to be as high as 5 million tonnes. Losses due to rots affect availability, food security and revenue of farmers and traders (Amusa *et al*., 2003). Rots of fleshy parts of plants develop as tissues are disintegrated by the action of microorganisms. Extra cellular enzymes are produced in advance of the bacterial cells

or fungal hyphae of pathogens that attack the tubers of yam. The affected tubers become hydrotic and soft, turn brown, emit offensive odour and exhibits a sharp demarcation between a healthy intact tissue and a diseased tissue (Aidoo, 2007). Yams are subject to several diseases caused by fungi, bacteria and viruses. Fungi, however, are the major causes of post harvest rots of yam tubers (Okigbo and Ikediugwu, 2000). The major fungal pathogens causing rot diseases in yams include *Aspergillus flavus* Lark ex Fr*., Aspergillus niger* Van Tiegh*, Botryodiplodia theobromae* Pat*, Fusarium oxysporum* Schecht ex Fr*., Fusarium solani* (Mart.) Sacc*., Penicillium chrysogenum* Thom*, Rhizoctonia* spp*, Penicillium oxalicum* Currie and Thom and *Rhizopus nodosus* N‟amyslowski (Okigbo and Ikediugwu, 2002; Okigbo and Emoghene, 2004). Suleiman (2010) has reported *Alternaria solani* isolated from rotting yam tubers whose pathogenicity has been proven and assessed. Ashraf *et al*. (2012) also have reported *Verticillium dahliae* isolated from related crop potato wilt in Pakistan.

Aboagye-Nuamah *et al*. (2005) have reported that nine fungal species including *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Fusarium culmorum, Fusarium oxysporum, Fusarium* spp, *Penicillium brevi-compactum, Penicillium* spp and *Rhizopus stolonifer* and a bacterium, *Erwinia carotovora* were identified to be associated with yam tuber rots in Ghana. Fungal pathogens causing rots in yam often gain entry into tubers through wounds caused by insects, nematodes or poor handling before, during and after harvest (Amusa *et al.*, 2003).

The field diseases are those diseases that cause economic damage to yam in the field from the seedling stage to the point of harvest, which are inflicted by pests, especially yam beetles (*Coleoptera* and mealy bug), nematodes and termites. Field symptoms on the leaves lesions were classified into either spots or blights on leaves yam (Morse *et al*., 2000). Previous work (Amusa and Ayinla, 1997) indicated that fungi associated complex field diseases of yam such as *Colletotrichum gloeosporioides* (leaves and stems), *Curvuleria pallescens* (leaves), *Curvularia eragrostides* (leaves), *Pestalotia* spp (leaves), *Sclerotium rolfsii, Colletotrichum graminicola, Botryodiplodia theobromae, Fusarium oxysporum* and *Macrophorria* spp and *Rhizoctonia solani* (leaves and stems) in Southwestern Nigeria. These associated fungi were also found to induce necrotic lesion of varying sizes on the leaves yam plant. On susceptible yam cultivars, symptoms

appeared at first as small dark brown or black lesion on the leaves, petioles and stems (Amusa and Ayinla, 1997).

3.9. Symptoms and infection of yam rot diseases

The storage diseases of yam can be categorized into three, based on the symptoms and infection of the causal agents (Amusa and Baiyewu, 1999). The first category is dry rot in which symptoms are vary with varying coloration, depending on the invading pathogen, the infected tissues become hard and dry. When tubers are infected with *Penicillium oxalicum* and *Penicillium cyclopium*, the tubers turn brown and then become hard and dry, maintaining their integrity, except when the tissues were invaded by *Sphaerostilbe marcescens.* Tissues invaded by *Sphaerostilbe marcescens* become covered with the greenish mycelia of the fungus. Tubers infected with *Aspergillus niger* and *Aspergillus tamari* turn brown with yellowish margin. *Rosellina bunodes* and *Botryodiploidia theobromae* have also been reported to cause dry black rots (Okigbo and Emoghene, 2004). Tubers infected by the two organisms first turn grey and then black. These tubers become pulverulent and break into small dry particles (Amusa *et al*., 2003). *Fusarium* spp were also reportedly associated with dry rot in yam tubers in Nigeria inducing pinkish with yellowish border on the infected tissues. The species of *Fusarium* implicated in dry tuber yam rot includes *Fusarium oxysporum, Fusarium moniliforme and Fusarium solani.* Tubers infected by *Sphaerostilbe repens* had reddish mycelia on the rotted part. Infected tissue was discolored brown and smelled fermented grains but maintained their integrity (Morse *et al*., 2000).

The second category of yam rot is soft rot in which infected tissues become soft ramified (cause to form branches; cause to spread and branch out) by the fungal mycelium. The causal fungi quickly ramified the tissue which turn brown and become soft and at times wet due to a rapid collapse of the cell walls. Fungi associated with this type of rot are *Rhizopus* spp, *Mucor circinelloides, Sclerotium rolfsii, Rhizoctonia solani* and *Armillariella mellea.* The third category is wet rot in which yam tuber is characterized by the oozing of whitish fluid out of infected tissues when pressed. This symptom is usually associated with the bacterium, *Erwinia carotovora* (Amusa and Baiyewu, 1999).

Table 4 Microorganisms found associated with the stored and marketed yam tubers obtained from the tropical forest region of Southwestern Nigeria and their pathogenicity on yam

Pathogens	Symptoms of infection	Pathogenicity
Botryodiplodia theobromae	Dry rot	+++
Aspergillus tamari	Dry rot	++
Penicillium oxalicum	Dry rot	+++
Penicillium cyclopium	Dry rot	+++
Penicillium italicum	Dry rot	+++
Fusarium oxysporum	Dry rot	++
Fusarium solani	Dry rot	++
Rhizopus nigricans	Soft rot	++
Sclerotium rolfsii	Soft rot	+++
Muccor circinelloides	Soft rot	+++
Erwinia carotovora	Wet rot	+++

++: Mildly pathogenic (> 10< 50 mm in diameter); +++: Highly pathogenic (≥50 mm in diameter); Source: Amusa and Baiyewu, 1999.

3.10. Storage and storage systems

Water loss from yam tubers continues during storage (Adesiyan and Odihirin, 1975). Yam rots usually start at maturity in the field due to entry of wounds by rot causing fungi or bacteria and progresses in storage. Regardless of the source of inoculum, most rot inducing fungal pathogens are unable to enter fleshy tissues except through open wounds. Tubers which are already attacked by rot pathogens when harvested are destroyed to a greater extent in storage. The rate at which this occurs depends upon the storage conditions (Osagie, 1992).

Adeniji (1970) has reported that considerable reductions in rots caused by fungal pathogens when tubers were stored in such a way that free air circulation was maintained compared to stock piling them on the floor of a shed. Coursey (1967b) has demonstrated that a temperature of 50^{o}C causes tubers to lose weight and rot much more quickly than those kept in shade. These conditions must be prevented in storage facilities. Optimum storage conditions for fungal growth

on yam were also reported to be 22-29^{o}C and 80% relative humidity and above (Ogundana *et al.,* 1970).

Causes of storage losses of yam tubers include sprouting and dehydration, transpiration, respiration, rot due to fungi, bacteria, insects and nematodes. Sprouting, transpiration and respiration are physiological activities which depend on the storage environment, mainly temperature and relative humidity (Alakali *et al*., 1995). These physiological changes affect the internal composition of the tuber and result in destruction of edible material, which under normal storage conditions can often reach 10% after 3 months, and up to 25% after 5 months of storage (Serge and Agbor-Egbe, 1996). All these reduce the overall quantity and quality of the tubers with food reserves being increasingly depleted by one or more of these causes.

3.11. Morphological and biological characters of fungal pathogens

3.11.1. Genus *Fusarium*

The genus *Fusarium* belongs to the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order. The genus comprises a high number of fungal species that can be plant pathogenic, causing diseases in several agriculturally important crops, including cereals, and can also be harmful for humans and animals. Many of them produce a wide range of biologically active secondary metabolites such as mycotoxins with extraordinary chemical diversity. The biological activity of *Fusarium* mycotoxins can be detrimental to plants (Desjardins, 2006). *Fusarium oxysporum, Fusarium moniliforme, Fusarium poa* and *Fusarium solani* are pathogenic species that causes rots of yam tubers (Okigbo and Ikediugwu, 2002).

The main approach for the *Fusarium* classification is still morphology and the primary trait for species to be placed in *Fusarium* genus is the occurrence of the asexual spores, the distinctive banana-shaped macroconidia, firstly diagnosed by Link (1809). *Fusarium* species produce three types of spores; macroconidia, microconidia and chlamydospores (Leslie and Summerell, 2006). An exact identification of *Fusarium* species on the basis of morphology requires the standardization of cultural methods like medium, temperature, light and duration of culture. As sporulation medium a low-nutrient agar like synthetic nutrient-poor agar (SNA) with 2 x 4 cm large piece of sterile filter paper on top of the cooled agar surface should be used (Nelson *et al*., 1983).

3.11.2. Genus *Alternaria*

Alternaria is a ubiquitous fungal genus that includes saprobic, endophytic and pathogenic species. Species of *Alternaria* are known as serious plant pathogens, causing major losses on a wide range of crops (Woudenberg *et al*., 2013). Among the yam rotting fungi associated with yam tuber *Alternaria solani and Alternaria alternata* are species that cause rotting to yam tubers (Suleiman, 2010). Morphological characteristics of conidia and conidiophores and sometimes host plant association, provide the major taxonomic criteria for delimitation of fungal species. Characteristics of the genus included the production of dark coloured phaeodictyospores in chains, and a beak of tapering apical cells (Simmons, 2007).

The fungus is readily cultured on artificial media such as V8 juice where it produces a deeply pigmented gray/black hairy colony. The mycelium is haploid and septate, becoming darkly pigmented with age. Sporulation in culture can be stimulated by exposure to fluorescent light. The conidia are composed of many cells and are produced on specialized fungal threads called conidiophores (Vloutoglou and Kalogerakis, 2000).

3.11.3. Genus *Verticillium*

Verticillium is a genus of fungi in the division Ascomycota, and are an anamorphic form of the *Plectosphaerellaceae* family (Kirk *et al*., 2008).The genus *Verticillium* is extremely heterogeneous and has a broad host range including trees, herbaceous plants, plantation crops and mushrooms extending from subtropical and tropical regions to cool and warm regions. The species infecting plants or living as saprophytes, *Verticillium albo-atrum*, *Verticillium dahliae*, *Verticillium tricorpus*, *Verticillium nigrescens*, *Verticillium nubilum* and *Verticillium theobromae* are still assigned to *Verticillium* (Pegg and Brady, 2002). Among the six species, *Verticillium dahlia* and *Verticillium albo-atrum* are the predominant plant pathogens worldwide; whereas the others are considered economically less important. Identification can be made by looking for one-celled conidia, hyaline round to ellipsoids which are formed at the tips of whorled branches (Barbara and Clewes, 2003). *Verticillium* symptoms may comprise wilting, chlorosis, stunting, necrosis and vein clearing. Brown vascular discoloration may be observed in stem tissue cross-sections. Pathogenicity of *Verticillium* species have been reported to produce

cell-wall-degrading enzymes and phytotoxins that all have been implicated in symptom development (Fradin and Thomma, 2006).

3.12. Management of fungal diseases

Yam disease control has been extensively studied and several measures have been recommended. Treatments of yam tubers with fungicides such as Benlate and Captan just after harvest have been recommended. The boring beetle attack on shoot and tubers can be controlled by application of granular Diazinon and Carbofuran (Amusa *et al*., 2003). Treatment of yam tubers with insecticide dust (Actellic 2% dust) will reduce insect pests attack and also ameliorate physical damages acquired during harvest, resulting in significantly fewer fungal lesions (Morse *et al*., 2000). Some biological control measures have been carried out, using microbes to control yam rot. Okigbo and Ikediugwu (2000) have showed that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the yam tuber.

3.12.1. Cultural practices

Cultural control consists of all methods a farmer can apply to control plant diseases except chemical, resistance breeding and biological control methods. Cultural practices for diseases control such as crop sequence, organic amendment, liming for pH adjustment, tillage and irrigation frequently influence disease through increasing or reducing the availability of various nutrients. Every cultural practice has a direct or more often indirect effect on the development of crop diseases (Harish *et al*., 2007).

Conventional tillage and sanitation of crop residues in the fall along with crop rotation, burying, burning and removal of postharvest residues are important cultural practices which can reduce primary inoculum. Earlier planting dates led to a marked delay in the development of anthracnose of *D. alata* compared with intermediate and later planting dates. The impact of anthracnose on early emerging yams was lower because the plants had time to establish a canopy before the onset of weather conducive to disease development or continuous rains. Mature leaves of yam are known to be more resistant to anthracnose than intermediate or juvenile ones (Sweetmore *et al*., 1994). Cultural practices such as intercropping, removing diseased plant parts, controlling weeds, selecting the best site, using proper plant spacing, proper fertilization,

avoiding overwatering and overhead irrigation can manage many foliar diseases (Ogali *et al*., 1991).

With the use sprout suppressants holding little promise at present as an economic means of prolonging the dormant period of yams (Osuinde *et al*., 2002); attention is beginning to be directed to temperature control as a practical alternative. By lowering the rate of biochemical and physiological reactions that ultimately lead to sprouting, low temperature able to prolong the storage life of yams by simply delaying sprouting (Okigbo and Osuinde, 2003). One of the most effective and simple means of reducing post harvest water and pathological losses of several root crops are by curing. The term curing as applied to root and tuber crops is used to indicate their controlled exposure to relatively high temperature and humidity for a short periods (about 24 hours) after harvest with intention of improving their subsequent storage life. Curing which was originally developed for potatoes has been successfully applied to yams (Okigbo, 2003). In general, the curing process involved exposure of the freshly harvested tubers to temperatures of 20-40^{o}C and relative humidity of 90-95% for 5-7 days. Basically, subjecting the tubers to a short period of high temperature and humidity encourages natural thickening of the tuber skin tissue and the healing of any surface wounds, thereby reducing the rate of water loss and preventing wound infection (Osuinde *et al*., 2002).

3.12.2. Biological control of fungal diseases

Biological control is the use of microbial antagonists to suppress diseases as well as the use of host specific pathogens to control diseases, insect pests and weed populations. The organism that suppresses the pathogen is referred to as the biological control agent. More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen (Kamal and Brian, 2006). Biological control is the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists (Baker and Cook, 1974). Biological control of plant diseases has proved to be durable in its effects and, has the advantage of not requiring repeated periodic applications as is the case with chemical pesticides. It is thus,

potentially better suited for use, particularly in developing economies (Okigbo and Ikediugwu, 2000).

Different biological control agents (BCAs) can be used for the control of plant diseases. These include fungi, bacteria and actinomycetes. The most important BCAs belong to the genus *Trichoderma* spp, *Bacillus* spp*, Pseudomonas* spp and *Streptomyces* spp **(**Suprapta, 2012). Biological control of plant pathogens is an attractive alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains. A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people, and the environment (Harman *et al*., 2004).

Manjula *et al*. (2005) reported that *Trichoderma harzianum* as a biocontrol agent against fungal pathogens of yam and cassava was effective in reduction of disease severity in greenhouse experiment. Also Begum *et al*. (2010) evaluated that *Trichoderma* strains under field conditions to assay their efficacy in suppressing *Alternaria* fruit rot disease and their applications improved both growth and yield compared to the treatment. Similarly, different *Trichoderma* isolates were evaluated against potato wilt disease (*Fusarium oxysporum*) under greenhouse condition. Potted plants treated with *Trichoderma* isolates plus *Fusarium oxysporum* showed lower disease incidence in comparison to *Fusarium* infested control (Ommati and Zaker, 2012).

3.12.2.1. Ecophysical characteristics of biocontrols versus pathogens

The BCAs exhibit different modes of action and hence, a good testing program should clarify all the mechanisms involved in the biocontrol activity of the BCAs. Apart from biocontrol ability, the BCAs possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Harman *et al*., 2004). These traits are useful for good BCA as they help in the establishment of the BCA in a given agro-ecological region.

3.12.2.2. Mechanisms of action of *Trichoderma* spp as biocontrol

Biological control involves the use of beneficial organisms, their genes, and/or products, such as metabolites that reduce the negative effects of plant pathogens and promote positive responses by the plant. A number of commercial products have been registered both at national and international levels based on different fungal and bacterial antagonists. These commercial products include, Biocon, Biogaurd, Ecofit, F-Stop, Soilgaurd, Trichodex with *Tricoderma* spp as active ingredient, and Mycostop, Rhizoplus Subilex utilizing various *Bacillus* species as active ingredient (Junaid *et al*., 2013).

The success of *Trichoderma* spp as a biocontrol agent is believed to involve various modes of action, including antibiotic production, secretion of lytic-enzymes, mycoparasitism, competition for space and nutrients, and induction of systemic resistance (Rocco and Perez, 2001). Both volatile and non-volatile antibiotics are known to be produced from *Trichoderma* species (Okigbo and Ikediugwu, 2000). Peptaibols (trichorizianines, trichokindins, trichorzins, trichorozins and harzianins) are a class of antibiotics produced by most species and strains of *Trichoderma* species. Peptaibols are thought to act on the membrane of the target fungus to inhibit membrane-associated enzymes involved in cell wall synthesis (Okigbo and Ikediugwu, 2000). The antibiotics trichodermin, trichodermol, harzianum A and harzianolide are also known to be produced from *Trichoderma viride* and other species of *Trichoderma* (Barbosa *et al*., 2001).

Studies have shown that mycoparasitic strains of *Trichoderma* produce a complex set of extra cellular enzymes including β-(1,3)-glucanase, chitinases, lipases and proteases when grown on isolated cell walls of pathogenic fungi (Cortes *et al*., 1998). Barbosa *et al.* (2001) reported that *T. viride* and *T. harzianum* secrete extra cellular cellulase. These lytic enzymes are probably responsible for hyphal lysis through the digestion of major cell wall components (Thangavelu *et al*., 2003).

Mycoparasitism occurs when one fungus exists in intimate association with another from which it derives some or all of its nutrients while conferring no benefit in return. The mycoparasitic relationship between *Trichoderma* spp and its potential host might involve biochemical and

physiological interactions that lead the microscopically visible phenomena of hyphal coiling, appressorium formation, penetration and cytoplasmic degradation (Cortes *et al*., 1998).

Competition is an indirect effect whereby pathogens are excluded by depletion of food bases or by physical occupation of sites. *Trichoderma viride* compete for the same niches with the pathogens (Okigbo and Ikediugwu, 2000). Thus, the rapid growth of *Trichoderma* gives it an important advantage in the competition for space and nutrients with plant pathogenic fungi (Barbosa *et al*., 2001).

3.12.3. Chemical control of fungal diseases

Chemical fungicides have been providing effective protection against many diseases but their application results in environmental pollution and emergence of resistant pathogen strains. In addition, chemical control which adds to the cost of production is often beyond the reach of the small farmers in developing nations (Basim *et al.,* 1999). The non-degradable components of these compounds have been accumulated over the years and entered the food chain, causing toxicity in animals (Harman *et al*., 2004). The environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people‟s attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticides use, and there must be judicious application in order to remove the most hazardous chemicals from the market (Rutherford, 2006).

Commonly used fungicides which are effective against *Fusarium* spp are: Carbendazim, Dithane M-45, Thiovit and Thiophanate-methyl significantly reduced the growth of *Fusarium oxysporum* (Abdul *et al.,* 2006). Aminoglycosides: amikacin, gentamicin, kanamycin A, kanamycin B, neomycin, and ribostamycin showed the best fungicidal activities against *Fusarium graminearum* and suppressed fungal infection (Yukie, 2008). Curzate (Copper Oxychloride 39.75% + Cymoxanil 4.2% WP) foliar spray at standard concentration (50-112 mg per liter) rates show good preventive and curative activity against downy mildew of grape and cucumbers, and late blight of potatoes and tomatoes. Sancozeb (mancozeb 80% WP) has been successfully used against a wide variety of diseases, particularly vegetables as foliar spray. It is popular with potato and tomato growers for blight control with the standard concentration of 0.2%. Had differential

response for control of *Alternaria solani*, *Alternaria alternata, Fusarium moniliforme* and *Fusarium solani* (Nene and Thapliyal, 1993).

Some soil borne root infecting fungi are difficult to eradicate because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Omer and Shahzed, 2007). Use of fungicides for the control of soil borne diseases is costly and also produces environment and health hazards to men and also adversely affects the beneficial microorganisms in the soil (Dluznieska, 2003). This has diverted the attention of plant pathologist towards alternate methods for the control of plant diseases.

The combined use of microorganisms and chemical fungicides has attracted much attention in order to obtain synergistic effects in an integrated pest control of soil borne diseases. Reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonists (Hjeljord and Tronsmo, 1998). Srinivas and Ramakrishnan (2002) reported that integration of microorganisms and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually.

For the purpose of *in vitro* evaluation of fungicides namely curzate and sancozeb, the methods of poisoned food technique was applied. After autoclaving, the medium was amended with different fungicide concentrations (Nene and Thapliyal, 1993). The inoculum was developed on Potato Dextrose Agar medium for both fungal pathogens and biocontrols, and they were inoculated on the media amended with different fungicide concentrations following the dual culture method (Hajieghrari *et al*., 2008).

4. METERIALS AND METHODS

4.1. Description of the study area

The study was conducted in Seka Chokersa, Jimma Zone of Oromia region, Ethiopia. The Jimma Zone is located 363 km southwestern 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 was amounted to 68%. Its agro-ecology was sub-humid tepid to cool mid high lands. Major soil types are upland with chromic nitosol and combisol, and bottom land with fluvisol.

4.2. Experimental site and sample collection

All the experiments were carried out in the Mycological Research Laboratory Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. Diseased samples of yam were collected from Seka Chokersa woreda, Jimma Zone, Ethiopia to isolate and characterize the fungal isolates from different parts of the plant (tubers and leaves) and were sampled from more than thirty yam cultivars planted in the fields. The number of samples collected was 37 (leaves=22 and tubers=15). These different parts of the plant samples were brought to mycological laboratory in clean plastic bags (envelopes). The samples were kept in a refrigerator at 4°C until isolation and further investigation/study.

4.3. Isolation of fungal pathogens of yam

The diseased leaves and tubers were cut into 1cm from the periphery of the infected rotten with a sterilized knife and washed in sterilized water for two minutes on separate plates in order to minimize surface contaminants. They 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 (DIFCO). 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 on to Potato Dextrose Agar which contained chloramphenichol with 2 mg (Aneja, 2005). The pure colonies were transferred on to PDA slants for further study.

4.4. Designation of isolates

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

4.5. Preparation of inoculum and pathogenicity test of isolates

The fungal isolates was 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 ^{o}C. The mycelial mats were filtered using Whatman No. 42 filter papers and thoroughly washed. The mycelial mats so harvested 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 employing Heamocytometer and the concentration of spore suspensions were adjusted to 1×10^{6} conidia/ml (Aneja, 2005).

Health looking yam tubers and leaves were washed with distilled water and sterilized with 70% ethanol to minimize surface contaminants 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 they were cut into 1cm square pieces which were used as a host for the test. A small amount of sterile distilled water was added to each Petri plates to make the environment moisture and pieces were put onto the slides. The samples were inoculated with two milliliter (2 ml) of the standardized spore suspension. The isolates were incubated at 25±1^{o}C for 20 days. They were examined daily for evidence of fungal growth, yam lesions, colonization, discoloration and mortality. Virulence was determined on the basis of leaves and tubers colonization and mortality as well as their symptoms. The controls were inoculated with 2 ml distilled sterilized 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).

4.6. 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 in order to make the environment conducive for the growth of the fungal isolates. With the help of a sterile scalpel 5 mm square block of the medium 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 cube. Thereafter, the Petri dishes were incubated at 25^{o}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 cover with cover slip after having a drop of lactophenol cotton blue. The slides were observed under low power (10×), middle (40×) power and high power objective (100×) using ocular and stage micrometer. The average measurements of spore were determined and shapes of the spores were recorded using Olympus microscope. Microphotograph was taken to show the typical spore morphology of the fungal isolates (Sangdee *et al*., 2011).

4.7. Identification of fungal pathogens

The fungal isolates from infected yam tubers and leaves such as pigment production, colony color, spore or conidia producing structures and spore shapes were recorded. Spore and mycelium characteristics were studied using the compound microscope 40× and 100× magnification. These characteristics were used in identifying the fungal isolates to the genus level, following standards described by Mathur and Kongsdal (2003) and Barnett and Hunter (1972).

4.7.1. Cultural and morphological characterizations

Four different solid media Potato Dextrose Agar Oxoid (PDA), Malt Extract Agar Oxoid (MEA), Czapeck Dox‟s agar Oxoid (CDA) and Sabouraud‟s dextrose agar Oxoid (SDA) were used for the study of growth characters. Each culture medium was prepared in one liter of distilled water and autoclaved at 121°C for 15 minutes. These were cooled and then poured into 90 mm Petri dishes for solidification. For the preparation of PDA, thirty nine grams (39 g) of potato dextrose agar; for MEA thirty one point eight gram (31.8 g) of malt extract agar; for CDA forty eight gram (48 g) of Czapeck Dox‟s agar; and for SDA sixty five gram (65 g) of Sabouraud‟s dextrose agar were dissolved in one liter distilled water by recommendations of manufacturers. Twenty five milliliter of each media was poured aseptically into Petri plates of 90 mm diameter. Five mm discs from an actively growing zone of seven days old fungal culture was placed upside down at the centre of the solidified medium and were incubated at 25±1^{o}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. The various cultural characters like, rate of growth (recorded within two days interval), type of margin, colony color, pigmentation and topography of the mycelium on different solid media were recorded on each medium by visual observation. 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.

4.8. Ecological studies of fungal isolates

4.8.1. 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 Petri plates containing PDA medium and incubated at 20^{o}C, 25^{o}C, 30ºC, 35^{o}C and 40^{o}C for eight days. Each experiment was done in three replications. The mycelial growth diameter was measured in millimeter for each treatment (Ramteke and Kamble, 2011).

4.8.2. Effect of hydrogen ion concentration on mycelial growth of fungal isolates

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 milliliters 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. The reaction of the medium was adjusted to the desired pH by adding 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 obtained. The cultures were filtered through Whatman No. 42 filter paper and the mycelial mats were dried at 45°C for 48 hrs. 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.

4.9. Nutritional studies of fungal isolates

4.9.1. 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; dextrose, sucrose, maltose and fructose (Otsuka *et al*., 1957). The 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 Barnet, 1951).). For this study sucrose was replaced by equivalent amount of the above mentioned carbon sources individually. That is, carbon sources were added to the Richard‟s agar medium at 21.053 g of carbon per liter of medium. The Richard‟s agar medium containing the above four carbon sources was autoclaved separately at 121^{o}C for 15 minutes. 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 center of each Petri plate and incubated for seven days at 25±1^{o}C. Three

replicates were used for each carbon source per isolates. Mycelial growth diameter of the fungal isolates was measured after seven days.

4.9.2. Effect of nitrogen sources on mycelial growth of fungal isolates

Similar procedures were carried for evaluating nitrogen sources using Richard‟s agar medium. The potassium nitrate in the Richard‟s medium was replaced by ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3), urea ($C H_4N_2O$) and considering potassium nitrate (KNO_3) as one treatment. The amount of nitrogen in the Richard‟s agar medium was replaced by equivalent amount of the above mentioned nitrogen sources (1.3855 g) individually. The Richard‟s agar medium containing the above nitrogen sources were autoclaved separately at 121 ^oC for 15 minutes. 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 center of each Petri plate and incubated for seven days at 25±1^oC. For each treatment, three replications were maintained for each nitrogen source per fungal test pathogens. Mycelial growth diameter of the fungal isolates was measured after seven days.

4.10. Combined effect of fungicide and antagonistic *Trichoderma* against fungal pathogens

4.10.1. *In vitro* evaluation of antagonistic activity of *Trichoderma* species

Two species of *Trichoderma (Trichoderma harzianum* and *Trichoderma viride)* were used to evaluate the antagonistic potentials against the fungal isolates and they were designated as AUT1 and AUT2, respectively. The culture was obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University which were isolated from the soil samples collected from Gera, Gomma, Mana, Kossa and Seka Chokersa woredas of Jimma Zone by Yonas Urbanos (2010). Dual culture method (Hajieghrari *et al*., 2008) was employed to evaluate the antagonistic potential of AUT1 and AUT2. The test fungal isolates were inoculated 12 hours prior to the placement of the *Trichoderma* spp to establish the growth of the test fungus because of *Trichoderma* spp are fast growers and occupied the space. A 5 mm diameter mycelial disc from the periphery of 7 day old culture of biocontrol was placed on the

opposite side of the fungal isolates on PDA. The experiment was arranged in three replicates. Additional plates having only the test fungal isolates were used as control. All plates were incubated at 25±1 ^{o}C. They were incubated for eight days. Radial growth reduction (percentage of inhibition) was calculated according to Montealegre *et al.* (2003) in relation to growth of the control by the following formula.

$$\text{Percent of inhibition} = \frac{(C-T)}{C} \times 100$$

Where C is radial growth measurement of the fungal isolates in the control plates and T is radial growth of the fungal isolates in the experimental plates. The inhibition was recorded in the form of overgrowth of the fungal isolates by the *Trichoderma* species.

4.10.2. *In vitro* evaluation and testing of fungicides against fungal pathogens

4.10.2.1. The poisoned food technique

The purpose of this experiment was to evaluate the efficacy of curzate and sancozeb fungicides at different concentrations against the fungal pathogens which were available currently on market to control pathogens. Evaluation and testing the effect of fungicides against the fungal isolates was employed according to Nene and Thapliyal (1993). The fungicides used were sancozeb and curzate. Sancozeb and curzate fungicides were obtained from Mycology Laboratory Research, Addis Ababa University. The fungicide concentrations were prepared as follows, if the formulated product (fungicide) has, 50% active ingredient, for 1 ppm solution 2 mg of the formulated product should be dissolved in a liter of solvent (Nene and Thapliyal, 1993). Therefore, curzate (Copper Oxychloride 39.75% + Cymoxanil 4.2%) has 43.95% WP, for 100 ppm solution 0.175 g, 200ppm (0.35 g), 400ppm (0.7 g), 600ppm (1.05 g), 800ppm (1.4 g) and 1000ppm (1.75 g) was added in a liter of solvent. For preparation of sancozeb (mancozeb 80% WP) 0.32 g, 0.64 g, 1.28 g, 1.92 g, 2.56 g and 3.2 g were used for 100, 200, 400, 600, 800 and 1000ppm, respectively and dissolved in a liter of distilled sterilized water. The fungicides were added to the autoclaved PDA medium (to prevent denaturation of the fungicides) cooled to 45°C with the amount of 2 ml per plate, so that the required concentrations were obtained. Triplicate culture plates, each containing 20 ml of the test medium, were used to test each fungal isolates at different concentration. Potato dextrose agar (PDA) plates inoculated with fungal

isolates without fungicide were used as control.

Mycelial plugs of fungal isolates, 5 mm in diameter were cut from 7 days actively growing margins of the fungal pathogen culture by sterile cork borer and transferred aseptically into the center of the Petri dish containing PDA medium with different concentrations of fungicide. Inoculated plates were incubated at 25^{o}C for 10 days. Growth of fungal isolates at each concentration was determined by measuring mycelial growth diameters in two perpendicular directions on each culture plate. Measurements were averaged in triplicates, and the diameters of the plugs used to inoculate the plates were subtracted from each measurement. The relative growth reduction for each fungicide was calculated by the equation below.

$$L = \frac{(C - T)}{C} \times 100$$

Where L is percent of inhibition; C is radial growth of the fungal isolates in control; T is radial growth of the fungal isolates in the presence of the fungicides (Rita and Tricita, 2004).

4.10.3. Tolerance of BCAs (*Trichoderma* spp) to fungicides

The fungicide concentrations were prepared by the poisoned food technique as previous according to Nene and Thapliyal (1993). In this technique, the growth medium was poisoned with sancozeb and curzate fungicides. The fungicide concentrations of 100, 200, 400, 600, 800, and 1000 ppm for both curzate and sancozeb were prepared on which on which the *Trichoderma* spp (AUT1 and AUT2) were screened for their tolerance to fungicides. Three triplicates were maintained for each treatment. Percentage inhibition of radial growth was calculated following the formula suggested by Rita and Tricita (2004):

$$L = \frac{(C - T)}{C} \times 100$$

Where L is mean inhibition percent of radial mycelial growth; C is radial growth measurement of the *Trichoderma* spp in control; T is radial growth of the *Trichoderma* in the presence of fungicides.

4.10.4. Combination of *Trichoderma* spp with fungicides against fungal pathogens

The combined use of *Trichoderma* spp (AUT1 and AUT2) and fungicides were applied under *in vitro* condition by the method of Nene and Thapliyal (1993). In this technique, the growth medium was poisoned with fungal toxicants. The fungicides used were sancozeb and curzate. The combinations of fungicides were done separately with each biocontrols. The fungicide concentrations of 600 ppm/lt for curzate and 400 ppm/lt for sancozeb were prepared on which both the test fungal isolates and antagonists can grow and added to the autoclaved PDA medium after cooling to 45^{o}C so that the required concentration was obtained for both fungicides. Triplicate culture plates, each containing 20 ml of the test medium was poured and after solidification of the medium, the test fungal isolates were inoculated 12 hours prior to the placement of the *Trichoderma* spp to establish the growth of the test fungal isolates following the dual culture method. Potato dextrose agar plates without BCAs and fungicides were used as a control. The growth of fungal isolates at 600 ppm/lt and 400 ppm/lt fungicides together with biocontrol agents were determined by measuring mycelial growth diameters and percentage inhibition of radial growth was calculated following the formula suggested by Rita and Tricita (2004):

$$L = \frac{(C - T)}{C} \times 100$$

Where L is mean inhibition percent of radial mycelial growth; C is radial growth measurement of the test fungal pathogens in control; T is radial growth of the test fungal pathogens in the presence of *Trichoderma* spp and fungicides.

4.11. Methods of data analysis

The statistical analysis of mycelial growth diameters of fungal isolates on different media, temperature, pH, carbon, nitrogen and percent of inhibition were tested. Mean comparisons of pathogens based on different parameters were conducted using the procedures of SPSS statistical analysis software version 16. Mean separation was determined according to Duncan‟s multiple range test ($P<0.05$).

5. RESULTS

5.1. Isolation and identification of fungal isolates

In the present study, ten different fungal isolates were isolated from infected leaves and tubers of yam from Seka Chokersa woreda, Jimma zone, Ethiopia. Among these isolates, five isolates (based on its pathogenicity test) were tested to confirm that the fungal isolates were pathogens of yam by the pathogenicity test study. These isolates were selected based on their pathogenicity test. The fungal isolates were purified through repeated sub-culturing and identified to genus level based on cultural, morphological and spore characteristics. These fungal isolates were labeled as AUF1 (*Fusarium* spp), AUA1and AUA2 (*Alternaria* spp) and, AUV1 and AUV2 (*Verticillium* spp).

5.2. Pathogenicity test

The pathogenicity test showed that all the five fungal isolates (AUF1, AUA1, AUA2, AUV1 and AUV2) were conformed their pathogenic effect and hence caused rot lesions, discoloration, colonization and mortality on healthy looking yam leaves and tubers after twenty (20) days of inoculation (Table 5). 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 5). The re-isolated fungal pathogens were similar with that of the original isolates obtained from infected leaves and tubers culturally and morphologically.

Table 5 Per cent of tissues infections and sporulation on yam leaves and tubers by fungal isolates

Fungal isolates	Per cent of tissues 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, ++=moderately pathogenic, +++= highly pathogenic

5.3. Characterization of the pathogens

5.3.1. Cultural characteristics

With regards to colony colors, the fungal isolates showed pink (AUF1), dark gray (AUA1), gray (AUA2), white (AUV1 and AUV2) on the front sides and black color on reverse sides of all growth media with mycelial topography of flat and raised fluffy growth, except AUF1 showed white color on both sides of the CDA medium (Table 6). Isolates AUF1, AUA1 and AUA2 showed raised fluffy growth, whereas AUV1 and AUA2 showed flat mycelium growth on all the media. There was no significant difference on pigmentation of AUA1 and AUA2 with dark gray and gray on the front sides while black color was exhibited on the reverse sides of all media, respectively (Table 6). With the same pattern AUV1 and AUV2 revealed white color on the front side and black color on the reverse side of all the media tested, respectively (Table 6).

Table 6 Effect of different culture media on cultural characteristics of 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 all of them supported the growth of all the isolates (Table 7). 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) and with minimum mycelial growth diameter of (61.33±3.21 mm) by AUA2 on MEA medium (Table 7). 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. 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. The maximum mycelial growth diameter of the isolates was supported by PDA followed by MEA and CDA, whereas the minimum growth diameter was recorded on SDA for all the fungal isolates (Table 7). The means of mycelial growth diameter of the isolates were significantly ($p<0.05$) different at 5% level of significance.

Table 7 The effect of different solid media on growth diameter of fungal isolates after 10 days of incubation

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

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.

5.3.2. Conidial characteristics

Measurements of the size of conidia (length and width) of the fungal isolates were shown in Table 8. The lengths and widths of fifty (50) randomly chosen conidia were measured under high power objective (100×) microscope for each fungal isolates. 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 8). 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 conidial features of each fungal isolates were recorded to confirm the test fungal isolates (Table 8). The of conidia, *Fusarium* spp (AUF1) has banana-shaped conidia with foot cells in sporodochia and three septate spores were commonly found. Likewise, the conidia of *Alternaria* spp (AUA1 and AUA2) were muriform shape and light brown colour (Figure not shown). The conidial shapes of *Verticillium* spp (AUV1 and AUV2) were hyaline, ovoid-ellipsoidal to sub-cylindrical shape produced at the apices of phialides.

Table 8 Conidial size (length and width) and other features of fungal isolates after twenty days of incubation at 25^{o}C

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

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

5.4. 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 9). The mean colony diameter of all the fungal isolates on solid medium was maximum within at temperature range of 25-30°C (Table 9). Also the result revealed that the mean mycelial growth diameter of fungal isolates on solid medium was maximum at 25°C in all the fungal isolates with the exception of AUA1 followed by 30°C (Table 9).

Table 9 The effect of different temperature on the fungal isolates after eight days of incubation

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

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 and mycelial diameter of 63.00±1.00 mm and 54.50±2.50 mm were recorded for the isolate AUV2 at temperature ranges of 25-30°C on Potato Dextrose Agar medium, respectively (Table 9). Of these optimum temperature ranges, the lowest mean mycelial growth were recorded by AUA2 (33.50±3.50 mm) followed by AUA1 (37.50±2.50 mm). Temperatures below 25°C and above 30°C reduced the growth of fungal isolates in some extent (not suitable for the growth of fungal isolates). However, in the present study no growth was observed at 40°C for all the fungal isolates as shown in Table 9. There were significant ($p<0.05$) interactions between fungal isolates and temperature levels.

5.5. The effect of different pH on mycelial growth of fungal isolates

All the fungal isolates grew in 3.0-8.0 range of pH values tested (Table 10). However, the dry mycelial weight was different among the 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) with the same isolate. Similarly, at pH 6.0 maximum biomass was measured (693.9±3.3 mg) followed by 693.2±1.2 mg and 624.5±4.0 mg for AUA1, AUV1 and AUF1, respectively which are not significantly different ($p<0.05$). Also at pH 7.0 maximum (356.4±1.1 mg) dry mycelial weight was measured for AUA2 and 693.4±4.9 mg in case of AUV1 at pH 5.0 (Table 10).

Table 10 The effect of different pH on the dry mycelial weight of the 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	$236.3±4.1^{b}$	$550.1±3.6^{a}$	$204.6±3.6^{c}$	$486.2±4.1^{d}$	$104.6±2.4^{e}$
4.0	$494.1±3.4^{e}$	$574.9±4.3^{b}$	$227.3±3.0^{a}$	$530.1±4.9^{d}$	$167.4±2.4^{c}$
5.0	$585.7±5.4^{a}$	$682.5±2.1^{c}$	$247.2±3.5^{b}$	$693.4±4.9^{c}$	$695.4±2.8^{c}$
6.0	$624.5±4.0^{b}$	$693.9±3.3^{c}$	$287.8±2.6^{a}$	$693.2±1.2^{c}$	$697.6±1.6^{c}$
7.0	$471.2±2.6^{f}$	$592.7±2.0^{a}$	$356.4±1.1^{c}$	$372.3±1.5^{d}$	$294.6±1.8^{b}$
8.0	$417.1±1.3^{a}$	$521.0±1.1^{b}$	$272.6±2.9^{c}$	$314.1±1.4^{d}$	$281.5±0.9^{e}$
Mean±SD	471.5±37.9	602.5±19.4	266.0±14.7	514.9±43.5	373.5±71.5

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.

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

The growth of the fungal isolates were different among fungal isolates and among different carbon sources ($p<0.05$) (Table 11). All the carbon sources were supported the growth of fungal isolates even though their growth was different on different carbon sources (Table 11). 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). The 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±0.51mm), 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 11 The effect of four different carbon sources on the mycelial growth of the 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±1.84^{b}	74.86±4.44^{a}	64.36±2.20^{b}	63.93±1.70^{a}
AUA1	48.16±2.24^{a}	54.26±1.35^{b}	49.00±1.80^{c}	53.13±2.19^{b}
AUA2	63.13±1.70^{d}	77.83±1.77^{c}	66.16±1.55^{d}	74.43±1.60^{c}
AUV1	90.00±0.00^{c}	89.66±0.57^{d}	80.33±0.77^{a}	89.70±0.51^{d}
AUV2	64.33±1.45^{d}	81.96±1.90^{e}	77.20±1.55^{a}	78.13±1.26^{e}
Mean± SD	68.10±14.50	73.68±12.86	69.75±12.26	71.87±12.97

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.

5.7. 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 presented in Table 12. 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). Potassium nitrate was

found to be good source of nitrogen for AUF1 (89.33±1.15 mm), AUA1 (43.33±3.05 mm), AUV1 (88.33±1.15 mm) and AUV2 (86.00±2.64 mm). The 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 fungal isolates.

Table 12 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±2.51^{c}	54.33±1.52^{b}	89.33±1.15^{c}	63.00±3.00^{c}
AUA1	42.66±3.05^{b}	36.33±2.08^{a}	43.33±3.05^{b}	23.00±2.64^{a}
AUA2	26.00±1.00^{a}	69.00±1.00^{c}	54.66±1.52^{a}	39.66±2.08^{d}
AUV1	73.33±2.08^{d}	72.66±2.08^{d}	88.33±1.15^{c}	26.66±3.05^{a}
AUV2	61.33±1.52^{c}	55.33±2.08^{c}	86.00±2.64^{c}	57.00±2.00^{b}
Mean±SD	53.20±17.48	57.53±13.38	72.33±20.17	41.87±16.62

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.

5.8. Biocontrol agents and fungicides activities against the mycelial growth of fungal isolates

5.8.1. *In vitro* evaluation of antagonistic activity of *Trichoderma* species

In dual culture test, each *Trichoderma* spp (AUT1 and AUT2) demonstrated different degrees of inhibition against the growth of fungal isolates and overgrew the fungal mycelial growth and showed significant differences in comparison to control after seven days of incubation at 25 ^{o}C (Table 13). Similar growth inhibition was recorded for both antagonistic over the mycelial growth of AUA2 with AUT1 (76.95%) and AUT2 (75.75%) (Table 13). AUV2 showed

58.33±2.08 mm radial growth extension in the control and 14.25±1.00 mm towards the antagonistic fungus AUT2 with percentage inhibition of 75.57%. AUT2 revealed the best performance where the mycelial growth of the fungal isolate AUF1 was 14.67±2.52 mm compared to 57.67±2.52 mm in control treatment. AUT2 (antagonistic fungus) suppressed the mycelial growth AUF1 by 74.62% whereas AUT1 showed 72.15% of inhibition. AUT1 (62.35%) followed AUT2 (68.82%) in performance against AUA1. From the comparison of means of means, AUT2 (73.30%) was more effective than AUT1 (69.12%) in terms of percentage inhibition of fungal isolates as presented in Table 13.

Table 13 *In vitro* evaluation of antagonistic activities of *Trichoderma* spp against mycelial growth of fungal isolates

Fungal isolates	**Control growth (mm)**	***Trichoderma harzianum* (AUT1)**		***Trichoderma viride* (AUT2)**	
		Growth of pathogen (mm)	% of inhibition	Growth of pathogen (mm)	% of inhibition
AUF1	57.67±2.52^{a}	16.00±2.00^{a}	72.15^{a}	14.67±2.52^{b}	74.62^{b}
AUA1	67.67±2.52^{c}	24.81±2.00^{b}	62.35^{b}	20.43±1.53^{a}	68.82^{c}
AUA2	50.67±3.06^{b}	11.67±0.58^{c}	76.95^{a}	12.33±2.08^{c}	75.75^{d}
AUV1	86.00±2.00^{d}	34.03±1.53^{d}	60.42^{b}	25.59±2.65^{d}	70.24^{c}
AUV2	58.33±2.08^{a}	15.33±1.53^{a}	73.72^{a}	14.25±1.00^{b}	75.57^{b}
Mean± SD	64.06±12.82	14.26±2.49	69.12	13.13±2.32	73.30

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.

5.8.2. *In vitro* evaluation of fungicides on mycelial growth of fungal isolates

In vitro evaluation of the inhibitory effect of two fungicides curzate (43.95% WP) and sancozeb (80% WP) on the test fungal isolates were evaluated using different concentrations of 100, 200, 400, 600, 800 and 1000 ppm (Table 14 and 15). There were significant ($p<0.05$) differences among the concentrations of curzate and sancozeb on the mycelial growth inhibition of fungal isolates on the growth medium (Table 14 and 15). But a significant ($p<0.05$) difference was observed between concentrations of curzate and the mycelial growth of all the fungal isolates for up to ten days of incubation at 25°C with the exception of AUA2 in which the complete inhibition was observed at the concentrations of 800 and 1000 ppm. Also significant ($p<0.05$) difference of inhibition was recorded for sancozeb fungicide with regard to concentration ranges from 100-1000 ppm in all the fungal isolates.

The result of Table 14 and 15, the response of individual fungal isolates to the two fungicides at different concentration was presented. Relatively maximum percent of inhibition (100%) was recorded between 400-1000 ppm against all the fungal isolates, except that AUA1 showed growth throughout the concentrations of sancozeb compared to the rest of fungal isolates. The highest (75.7%) percentage inhibition of mycelial growth of the isolate was displayed against AUA2 at the lowest concentration of 100 ppm by curzate and 87.6% in case of sancozeb with complete inhibition between 400-1000 ppm (Table 14 and 15). Likewise, AUV1 and AUV2 were completely inhibited (100%) by sancozeb with the range of concentrations between 600-1000 ppm. The same pattern of inhibition was displayed on AUF1 at the concentrations of 800 and 1000 ppm by sancozeb.

Table 14 *In vitro* evaluation of curzate at different concentrations on mycelial growth of fungal isolates after ten days of incubation at 25 ^{o}C on PDA

Concentration (ppm)	**Mean inhibition percentage of fungal isolates**					Mean±SD
	AUF1	AUA1	AUA2	AUV1	AUV2	
100	37.8^{d}	42.2^{g}	75.7^{d}	24.4^{f}	20.7^{e}	40.1±3.8
200	40.0^{d}	47.8^{f}	81.1^{c}	39.6^{e}	37.4^{d}	49.1±4.1
400	44.2^{c}	55.9^{e}	84.2^{c}	47.3^{d}	46.7^{c}	55.6±5.6
600	48.9^{b}	63.0^{d}	90.1^{b}	62.2^{c}	57.2^{b}	64.2±3.9
800	59.6^{a}	69.6^{c}	100^{a}	71.8^{b}	65.4^{a}	73.2±6.2
1000	63. 3^{a}	76.9^{b}	100^{a}	79.0^{a}	69.6^{a}	77.7±8.2
Control	62.7^{e}	46.0^{a}	50.7^{e}	87.3^{g}	53.7^{f}	60.1±4.1
Mean±SD	50.8±4.1	57.3±4.9	83.1±6.4	58.8±8.5	50.1±6.3	70.4±5.1

Each value is an average of three replicates. Means followed by the same letters within a column are not significantly ($p<0.05$) different, according to Duncan‟s multiple range test. Control was measured in mm.

Table 15 *In vitro* evaluation of sancozeb at different concentrations on mycelial growth of fungal isolates after ten days of incubation at 25 ^{o}C on PDA

Concentration (ppm)	**Mean inhibition percentage of fungal isolates**					Mean±SD
	AUF1	AUA1	AUA2	AUV1	AUV2	
100	61.2^{a}	67.4^{a}	87.6^{a}	85.9^{b}	77.1^{c}	75.8±6.1
200	63.3^{a}	75.4^{b}	92.1^{b}	89.7^{a}	78.4^{c}	79.7±6.5
400	69.2^{b}	80.4^{c}	100^{c}	90.1^{a}	81.4^{c}	84.2±6.4
600	71.3^{b}	84.1^{d}	100^{c}	100^{d}	100^{b}	91.1±7.3
800	100^{c}	86.0^{d}	100^{c}	100^{d}	100^{b}	97.2±2.5
1000	100^{c}	89.1^{d}	100^{c}	100^{d}	100^{b}	97.8±2.1
Control	62.7^{d}	46.0^{e}	50.7^{f}	87.3^{c}	53.7^{a}	60.1±7.4
Mean±SD	75.3±6.5	75.4±5.6	90.1±6.8	93.2±2.4	84.3±6.4	83.7±2.7

Each value is an average of three replicates. Means followed by the same letters within a column are not significantly ($p<0.05$) different, according to Duncan‟s multiple range test. Control was measured in mm.

Results of Table 16 and 17 showed that, *Trichoderma* spp, AUT1 and AUT2 were screened for tolerance to fungicides like curzate and sancozeb. Incorporation of curzate and sancozeb in growth medium did not affect the growth of *Trichoderma* spp instead fungicides favored the growth of antagonistic fungi at lower concentrations of 100 and 200 ppm. However, by increasing the fungicidal concentrations to 400 and 600 ppm, the antagonists tolerate the fungicides to some extent and reduced slightly at higher concentrations of 800 and 1000 ppm compared to control. The highest (74.1%) per cent of inhibition was recorded at high concentration of 1000 ppm for curzate and (78.9%) for sancozeb fungicides. From comparison of means of means, AUT1was more inhibited than AUT2 by both fungicides. AUT2 was more tolerate both fungicides than AUT1 (Table 16 and Table 17).

Table 16 Screening of *Trichoderma* spp for tolerance to curzate at different concentration after seven days of incubation at 25 ^{o}C

Concentration (ppm)	***Trichoderma harzianum* (AUT1)**		***Trichoderma viride* (AUT2)**		Mean±SD
	Growth (mm)	% inhibition	Growth (mm)	% inhibition	
100	87.0±0.57^{a}	3.3^{b}	88.0±0.57^{f}	2.2^{a}	45.1
200	71.0±0.57^{b}	21.1^{c}	81.0±0.57^{e}	10.0^{b}	45.7
400	62.6±0.66^{c}	30.4^{d}	71.0±0.57^{d}	21.1^{c}	46.2
600	44.3±0.57^{d}	50.7^{e}	42.3±1.20^{c}	52.9^{d}	47.5
800	31.0±0.57^{e}	65.5^{f}	31.0±0.57^{a}	65.5^{f}	48.3
1000	23.3±0.88^{f}	74.1^{a}	28.6±0.88^{b}	68.1^{e}	48.4
Control (mm)	90.0±0.0^{g}	0.0^{g}	90.0±0.0^{f}	0.0^{a}	90.0
Mean±SD	53.2±5.43	40.84	57.0±5.80	36.7	53.02

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

Table 17 Screening of *Trichoderma* spp for tolerance to sancozeb at different concentration after seven days of incubation at 25 ^{o}C

Concentration (ppm)	***Trichoderma harzianum* (AUT1)**		***Trichoderma viride* (AUT1)**		Mean±SD
	Growth (mm)	% inhibition	Growth (mm)	% inhibition	
100	85.0±0.57^{f}	5.5^{b}	88.0±0.57^{e}	2.2^{a}	45.2
200	66.6±0.88^{e}	25.9^{c}	71.6±0.88^{d}	20.3^{b}	46.1
400	43.0±0.57^{d}	52.2^{d}	43.0±1.52^{c}	52.2^{c}	47.6
600	30.0±0.33^{c}	66.3^{e}	33.0±1.15^{b}	63.3^{d}	48.2
800	22.0±0.57^{b}	75.5^{f}	26.6±0.88^{a}	71.4^{e}	48.6
1000	19.0±0.57^{a}	78.9^{g}	25.6±0.33^{a}	70.3^{e}	48.7
Control (mm)	90.0±0.0^{g}	0.0^{a}	90.0±0.0^{e}	0.0^{a}	47.4
Mean±SD	50.8±5.85	50.7	54.0±5.75	46.7	47.4

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

5.8.3. *In vitro* combination *Trichoderma* spp with fungicides against fungal isolates

The *in vitro* studies of combined use of biocontrols (AUT1 and AUT2) with chemical fungicides (curzate at 600 ppm and sancozeb at 400 ppm) against the fungal isolates revealed that it was more efficient than fungicide (Table 14 and 15) and fungal antagonists (Table 13) individually. At these concentrations (400 ppm and 600 ppm), the fungal antagonists were 50% compatible with both fungicides (Table 18). Study on combined efficacy of biocontrols with fungicides in response to individual isolates revealed that the highest percentage inhibitions of mycelial growth were displayed as 85.6%, 79.7%, 87.5%, 89.3% and 80.2% when AUT2 was combined with sancozeb against AUF1, AUA1, AUA2, AUV1 and AUV2, respectively (Table 18). In the same pattern, the lowest percent of inhibition of mycelial growth of 77.6% and 71.1% was displayed when AUT1 was combined with curzate for AUV2 and AUA1, respectively, whereas AUT2 with curzate recorded the lowest inhibitions percentage against AUA1 (76.0%). AUV2 revealed the lowest per cent of inhibition of 78.7% when AUT1 combined with sancozeb.

Similarly, the lowest percent (79.7%) of inhibition was exhibited for AUA1 when AUT2 was combined with sancozeb.

Table 18 *In vitro* evaluation of combination of fungicides with *Trichoderma* spp at a concentration curzate (C) 600 ppm and sancozeb (S) 400 ppm on the growth of fungal pathogens

Fungal isolates	**Control (mm)**	**Mean % inhibition of fungicide + biocontrol**				
		AUT1+ C	AUT1+ S	AUT2+ C	AUT2+ S	Mean±SD
AUF1	62.7^{c}	79.2^{b}	82.9^{a}	82.4^{a}	85.6^{a}	78.5±2.5
AUA1	46.0^{d}	71.7^{c}	78.9^{a}	76.0^{b}	79.7^{a}	70.4±3.4
AUA2	50.6^{c}	81.6^{a}	86.7^{b}	80.2^{a}	87.5^{b}	77.3±2.5
AUV1	87.3^{d}	85.5^{b}	84.7^{c}	87.1^{b}	89.3^{a}	86.7±3.5
AUV2	53.7^{a}	77.6^{b}	78.7^{b}	77.6^{b}	80.2^{b}	73.5±3.3
Mean±SD	60.1±4.3	79.1±5.3	80.6±3.2	80.6±4.1	84.4±3.9	77.2±3.0

Each value is an average of three replicates. Means followed by the same letters within a row are not significantly ($p<0.05$) different, according to Duncan‟s multiple range test**.**

6. DISCUSSION

In the present study, five pathogenic fungal isolates were isolated from infected yam and characterized on the basis of cultural, morphological and physiological characters. The evaluation of *Trichoderma* spp and fungicides were carried out to determine the percentage inhibition of the mycelial growth of pathogens. The fungal pathogens were identified as *Fusarium* spp (AUF1), *Alternaria* spp (AUA1 and AUA2) and *Verticillium* spp (AUV1 and AUV2) (Mathur and Kongsdal, 2003).

The data showed that AUA1 and AUA2 produced light brown and muriform shape conidia measuring about 30.5-40.47×10.9-17.37 µm and 27.3-42.5×11.6-16.9 µm, respectively (Table 8). Similarly, Ramjegathesh and Ebenezer (2012) reported that the length and width of conidia 30.99-42.47×11.9-17.37 µm in *Alternaria alternata* isolates. Likewise, AUF1 has banana-shaped conidia with foot cells in sporodochia and has three septate with conidial measurement of 19.0-26.2×3.2-5.8 µm. These characters are in agreement with Sunita (1999) who reported that such characters belong to *Fusarium oxysporum*. Smith (2007) also reported that the microconidia of *Fusarium oxysporum* were typically 25-35×3-5 µm wide, dorsi-venterally curved, sickle-shaped 3-5 septated. The range of conidial measurement of AUV1 and AUV2 were 3.5-11.2×1.8-3.8 µm and 2.5-9.0×1.25-3.2 µm, respectively with hyaline, ovoid-ellipsoidal shape produced at the apices of phialides. These conidial features are in agreement with the finding of Jabnoun-Khiareddine *et al*. (2010) who reported that the conidial measurement of *Verticillium tricorpus* was 3.75-6.25×2.5 µm on the study of morphological variability among the *Verticillium* species.

The conformation of pathogenicity studies indicated that the symptoms observed under artificial conditions similar to that of type of natural symptom noticed on yam plant. This result is similar to the report on fungi associated with storage decay of Nigerian yams (Adeniji, 1970). The inoculation and re-isolation experiments confirmed that all the five fungal isolates were associated with yam diseases and these fungi were capable to induce typical disease symptoms. Similarly, Noon and Calhoum (1981) reported on 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 colors for AUF1, AUA1, AUA2, AUV1 and AUV2 on PDA medium, respectively which was the same as the original culture (Table 6).

Optimized growth conditions (media preference, temperature, pH) of the pathogen would be used as a starting point for *in vitro* and *in vivo* evaluation of the efficacy of different fungicides against the fungal pathogens. The fungal pathogens which are used and the media components used are responsible for the mycelial growth and spore production. In the present study, the yam 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. Minimum growth of the mycelium was recorded on SDA for all fungal isolates. Thus, PDA medium was the best preferred medium for the growth of fungal isolates compared to the three media. Similarly, Kulkarni (2006) 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) 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 have resemblance with the results of the present study in which *Verticillium* spp attained maximum mycelial growth on PDA followed by MEA culture media.

From the present result, each fungal isolate had their temperature range for their growth and sporulation. The maximum mycelial growths of fungal isolates were obtained at 25^{o}C for all the fungal isolates with the exception of AUA1 which showed maximum mycelial growth (39.50±0.50 mm) at 30^{o}C. Thus, optimum temperature of fungal isolates ranged between 25-30^{o}C. Similarly, Imran Khan *et al*. (2011) indicated that fungal growth of *Fusarium* spp was best between 25^{o}C and 30^{o}C. Ramteke and Kamble (2011) 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 funding that all the fungal isolates did not grew 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) reported mycelial growth of *Verticillium dahliae* isolated from potato wilt at different temperature ranging from 18-30ºC and found that temperature 26ºC was the best suited for the mycelial growth of *Verticillium dahliae* which in 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. This is similar to the previous study where the most suitable pH level for growth of *Fusarium oxysporum* was 5.0 and 6.0 (Gangadhara *et al*., 2004). Similarly, Imran Khan *et al*. (2011) showed that optimum pH for growth of *Fusarium oxysporum* ranged from 6.5 to 7.0. With regard to the AUA1, the dry mycelial weight was maximum at pH 6.0 (693.9±3.3 mg). Hubballi *et al*. (2010) 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 was accumulated maximum dry mycelial weight of 356.4±1.1 mg followed by pH 6.0 (287.8±2.6 mg). Among the pH levels 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 (slows down) to the growth of fungal isolates. Similarly, Bhat *et al*. (2003) reported that the growth of *Verticillium* spp occur over a wide range of pH 4.5-7.0 and with the optimum at pH 5.0. Ashraf *et al*. (2012) also reported maximum growth of *Verticillium dahliae* causing wilt on potato 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 know their effect on the growth of the fungal isolates. From the carbon sources studied, significantly the 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) 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* spp.

With regards to the nitrogen sources, the 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 for all fungal isolates, except AUA2 with maximum growth of 69.00±1.00 mm on media amended with ammonium nitrate. Similarly, Ramjegathesh and Ebenezer (2012) 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) also reported that potassium nitrate was effective source for the growth of *Fusarium* spp.

In the present study, the results of dual culture revealed the rapid colonization of the medium by AUT1 and AUT2 (*Trichoderma* spp) were effective in controlling colony growth of fungal pathogens isolated from infected yam leaves and tubers. The present observations confirmed the previous findings of Okigbo and Ikediugwu (2000) who reported that *Trichoderma viride* controls post harvest rot of yams, presumably by direct parasitism and antibiotic production. Sahi and Khalid (2007) also reported that percent of inhibition of 62% and 36% for *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum,* respectively.

The data showed that both *Trichoderma* spp showed the best performance against AUA1 (62.35±1.33% and 76.95±0.52%) and AUA2 (68.82±1.53% and 75.75±1.71%) inhibition by AUT1 and AUT2, respectively (Table 13). This was due to *Trichoderma harzianum* attack, some *Alternaria alternata* hyphae exhibited morphological changes such as deformation, increase of cellular vacuolization, cell wall disintegration and dissolution of cytoplasm as reported by Sempere and Santamarina (2007). Similarly, Pandey (2010) reported that *Trichoderma harzianum* caused 67.07% inhibition of *Alternaria alternata*, while an inhibition of 66.67% was recorded by using *Trichoderma viride*. The result indicated AUT2 gave the highest inhibition percentage value of 75.57±0.63%, whereas AUT1 inhibited 73.72±1.27% inhibition after seven days of incubation against AUV2. Hanson (2000) reported that biological control of *Verticillium dahliae* Kleb. in cotton with a mixture of lignin and *Trichoderma viride*.

With regards to *in vitro* evaluation of fungicides curzate and sancozeb fungicides decreased mycelial growth of isolates compared to the control. Isolate AUA2 was found to be the most sensitive to both fungicides. Sancozeb was highly toxic to the fungal isolates tested compared to curzate. Therefore, sancozeb was effective and retained its efficacy even at the lowest

concentrations by achieving 100% complete inhibition over the control compared to curzate fungicide. Similarly, Nisa *et al*. (2011) also observed that from the study amongst the non-systemic fungicides, mancozeb was the most effective (14.20mm) in reducing mycelia growth of *Fusarium oxysporum.* Harish *et al*. (2007) reported that mancozeb (0.2%), main component of sancozeb was observed to be the most effective, which significantly reduced the spore germination of *Helminthosporium oryzae.*

With regard to the treatment of the pathogens with biocontrol agents and fungicides, AUT1 and AUT2 showed potential antagonistic activity against the fungal isolates of yam (Table 16). The data showed that no inhibition of mycelial growth was noticed at 100 ppm level of both fungicides compared to the control and a gradual increase was observed in percent of inhibition as the concentration increased, but inhibition was lower than that of sancozeb. As Papavizas (1985) reported that the differentiating response of antagonists to various fungicides might be due to their inherent resistance to the fungicides and their ability to degrade chemicals. Thus, the results of the present screening would help in the selection of biocontrol agents which can be used with reduced dose of selected fungicides (curzate and sancozeb) for the control of yam fungal isolates.

The data showed (Table 18) that at the concentrations 400 ppm and 600 ppm, the *Trichoderma* spp was 50% compatible with both fungicides. This showed that the antagonists were able utilize the fungicides as a source of nutrient, but above these concentrations it may weaken the efficacy of Trichoderm spp (AUT1 and AUT2). Likewise, below these concentrations, the test fungal pathogens compete with the antagonists for nutrient and space, because these concentrations were not significantly effective in inhibition of mycelial growth for both test fungal isolates and biocontrols.

The results showed (Table 18) that effects of antagonists and fungicides and the interaction effect between antagonists and fungicides were significant ($p<0.05$) against fungal isolates. The maximum percentage inhibitions of the mycelial growth of the five fungal isolates were recorded when AUT2 was integrated with sancozeb followed by combination of AUT1 with sancozeb. Therefore, combination of sancozeb fungicide with AUT1 and AUT2 was more effective than combination of curzate with AUT1 and AUT2 in terms of mycelial growth inhibition percentage (Table 18). The data clearly showed that integration of fungicides and biological treatments

against fungal isolates under *in vitro* condition displayed potential of inhibiting the mycelial growth when compared to the application of biological or chemical treatments alone (Table 18).

Similarly, Srinivas and Ramakrishnan (2002) have reported that integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually. Silimela and Korsten (2001) have reported that the efficiency of the biocontrol agent could further be improved when it was applied with the recommended fungicide and used at a lower concentration. Thus, the antagonistic potential of *Trichoderma* spp in terms of enhanced modes of action as increased hyper parasitism activity in the present study.

The present study implies that, all the fungal pathogens had their optimum temperature and pH ranges for their growth and reproduction. *In vitro* application of fungicides (curzate and sancozeb) was effective in controlling fungal pathogens, but they are not environmentally friendly for managing the pathogens. Therefore, rather than applying these chemicals, it is very important to use *Trichoderma* spp (AUT1 and AUT2) for effective management of fungal pathogens of yam since they do not have side effect on the environments. During this study, there were constraints like laboratory facilities. Due to this constraints, identification of fungal pathogens were done by using conventional techniques. However, keeping these constraints in view, it is very important if they were identified by using the application of molecular techniques in addition to the conventional identification techniques.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

- The *in vitro* pathogenicity test confirmed that the fungal isolates were pathogens of yam.
- 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 25-30 ºC.
- A pH value below 5.0 and above 7.0 was inhibitory to the growth of fungal isolates.
- From the nitrogen sources potassium nitrate was best utilized by all fungal isolates
- Both *Trichoderma* spp (AUT1 and AUT2) were effective in growth inhibition of fungal pathogens.
- With regards to efficacy of the fungicides tested, sancozeb was found to be effective in growth inhibition of fungal pathogens.
- Combinations of biocontrols with chemical fungicides at lower concentration were more effective than the individuals alone.

7.2. Recommendations

On the basis of the above conclusion, the following recommendations are being suggested:

- There is a need to undertake an intensive research for diseases of yam crops in all yam growing areas of Ethiopia and quantification of yield loss by pathogens.
- Media optimization is important to understand cultural characteristics of the pathogens, so that it is well to prefer the medium for the pathogens.
- *In vitro* and *in vivo* evaluation of more fungal, bacterial and actinomycetes antagonists against the fungal pathogens of yam should be carried in the future on a large scale under field conditions.
- Fungal BCAs which benefit industry can replace chemical use and standardization, preparation for fungicidal tolerant biocontrols urgently.

REFERENCES

Abang, M. M., Winter, S., Mignouna, H. D., Green, K. R. and Asiedu, R. (2003). Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. *Afr. J. Biotechnol.* **2**: 486-496.

Abdul, Q., Arain, M., Pathan, M., Jiskani, M. and Lodhi, A. (2006). Efficacy of different fungicides against *Fusarium* wilt of cotton caused by *Fusarium oxysporum*. *Pak. J. Bot*. **38**: 875-880.

Aboagye-Nuamah F., Offei, S. K., Cornelius, E. W. and Bancroft, R. D. (2005). Severity of spoilage storage rots of white yam (*Dioscorea rotundata* Poir.). *Annals of Appl. Biol.* **147:** 183-190.

Adeniji, M. O. (1970). Fungi associated with storage decay of yam in Nigeria. *Phytopathol.* **60**: 590-592.

Adesiyan, S. O. and Odihirin, R. A. (1975). Histopathology studies of the yam tuber (*Dioscorea rotundata* Roir) infected with *Scutellonena bradys* (Steiner& Hettew). *Int. Biodeterioration Bulletin* **11**: 48-55.

Adetuyi, F. O., Aladekoyi, G. and Adetuyi, O. O. (2010). The nutritional composition of cut surface of yam (*Disocorea rotundata*). *J. Sustain. Dev. Agric. Environ.* **5**: 108-116.

Afoakwa, E. O. and Sefa-Dedeh, S. (2002b). Changes in cell wall constituents and mechanical properties during post-harvest hardening of trifoliate yam *Dioscorea dumetorum* (Kunth) pax tubers. *Food Res. Int.* **35**: 429-434.

Aidoo, K. A. (2007). Identification of Yam Tuber Rots Fungi from Storage Systems at the Kumasi Central market. PhD Dissertation, University of Ibadan, Nigeria, submitted to Faculty of Agriculture, pp 18-19.

Akem, C. N. (1999). Yam die-back and its principle cause in the yam belt of Nigeria. *Pak. J. Biol. Sci*. **2**: 1106-1109.

Akissoe, N., Mestres, C., Hounhouigan, J. and Nago, M. (2003). Biochemical origin of browning during the processing of fresh yam (*Dioscorea* spp.) into dried product. *J. Agric. Food Chem.* **53**: 2552-2557.

Alakali, S.E., Obeta, O. and Ijabo (1995). Heat of respiration of yam tubers and its effects on heat load. *African Journal of Root and Tuber Crops* **1**: 31-35.

Albrecht, M. A. and McCarthy, B. C. (2006). Seed germination and dormancy in the medicinal woodland herbs *Collinsonia canadensis* L. (Lamiaceae) and *Dioscorea villosa* L. (*Dioscoreaceae*). *Flora* **201**: 24-31.

Amanze, N. J., Agbo, N. J., Eke-Okoro, O. N. and Njoku, D. N. (2011). Selection of yam seeds from open pollination for adoption in yam (*Dioscorea rotundata* Poir) production zones in Nigeria. *J. Plant Breeding and Crop Sci.* **3**: 68-73.

Amusa, N. A. and Ayinla, M. A. (1997). The effect of tecto (Thiabendazole) on the activities yam rot causing fungi and on sprouting of yam set. *Int. J. Trop. Plant Dis.* **14**: 113-120.

Amusa, N. A. and Baiyewu, R. A. (1999). Storage and market disease of yam tubers in southwestern Nigeria. *Ogun J. Agric. Res.***11**: 211-225.

Amusa, N. A., Adegbite, A. A, Muhammed, S. and Baiyewu, R. A. (2003). Yam diseases and its management in Nigeria. *Afr. J. Biotechnol.* **2**: 497-502.

Aneja, K. R. (2005). *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th ed. New Age International Publishers, New Delhi. Pp. 607.

Ashraf, A. R., Abbas, M. F. and Rehman, R. (2012). Isolation and identification of *Verticillium dahliae* causing wilt on potato in Pakistan. *Pak. J. Phytopathol.* **24:** 112-116.

Babaleye, T. (2003). "West Africa; Improving Yam Production Technology". ANB-BIA supplement Issue/Edition Nr 463.

Baker, E. F. and Cook, R. J. (1974). *Biological Control of Plant Pathogens*. W.H. Freeman and Co. Sanfransisco, Pp. 433.

Barbara, D. J. and Clewes, E. (2003). "Plant pathogenic *Verticillium* species: how many of them are there?" *Mol. Plant Pathol.* **4**: 297-305.

Barbaso, M.A.G., Rehn, K.G., Menezes, M. and Mariana, R.L.R. (2001). Antagonism of *Trichoderma species* on *Cladosporum herbarium* and their enzymatic characterization. *Braz. J. Microbiol*. **32**: 98-104.

Barnett, H. L. and Hunter, B. B. (1972). *Illustrated Genera of Imperfect Fungi*. 3rd ed. Burgess Publishing Company, Minneapolis, Minnesota, USA. Pp 241.

Basim, H., Ozturk, S. B. and Yegen, O. (1999). Efficacy of a biological fungicide (*Trichoderma harzianum Rifai T-22*) against seedling root rot pathogens (*Rhizoctonia solani*, *Fusarium* species) of cotton. GAP-*Environmental Symposium*. Sanliurfa. Turkey. Pp. 137-144.

Begum, F., Rahman, M. A. and Firoz Alam, M. (2010). Biological control of *Alternaria* fruit rot of chili by *Trichoderma* species under field conditions. *Microbiology* **38**:113-117.

Bhat, R. G., Smith, R. F., Koike, S. T. Wu, B. M. and Subbarao, K.V. (2003). Characterization of *Verticillium dahliae* isolates and wilt epidemics in pepper. *Plant Dis.* **87**: 789-797.

Brand-Miller, J., Burani, J. and Foster-Powell, K. (2003). The New Glucose Revolution - Pocket Guide to the Top 100 Low GI Foods. Marlowe and Company. Pp.158.

Cortes, C., Gutierrez, A., Olmedo, V., Inbar, J., Chet, I., and Herrera-Estrella, A. (1998). The extension of gene involved in parasitism by *Trichoderma harzianum* is triggered by a diffusible factor. *Mol. Gen. Genet*. **260:** 218-225.

Coursey, D. G. (1967a). Yams. Longmans, London. Pp 230.

Coursey, D.G. (1967b). Yam storage-1: A review of yam storage practices and of information storage losses. *J. Stored Prod. Res.* **2**: 229-244.

Dandge, V. S. (2012). Effect of nitrogen sources on the growth of different species of *Curvularia, Fusarium*, *Phoma* and *Botryodiplodia*. *J. Exp. Sci.* **3**: 24-27.

Desjardins, A. E. (2006). *Fusarium* mycotoxins*: Chemistry, Genetics and Biology.* American Phythopathological Society Press. St. Paul, Minnesota, USA. Pp 107.

Dluzniewska, J. (2003). Reaction of fungi of *Trichoderma* genus to selected abiotic factors. *Elec. J. Polish Agr. Uni. Agron*. **6**: 15-21.

Egesi, C. N., Onyeka, T. J. and Asiedu, R. (2007). Severity of anthracnose and virus diseases of water yam (*Dioscorea alata* L.) in Nigeria I: effects of yam genotype and date of planting. *Crop Prot.* **26**: 1259-1265.

Eka, O. U. (1985). The Chemical Composition of Yam Tubers, **In**: *Advances in Yam Research*, pp. 61-83, (Osuji, G., ed.). Frontline Publishers, Enugu-Nigeria.

FAO. (1975). FAO Annual report. Food and Agricultural Organization Production Year Book. FAO, UN, Rome. http://www.fao.org

FAO. (1987). "Formulation Reports: Roots and Tubers Expansion Programme". FAO, UN, Rome, Italy. http://www.fao.org

FAO. (2002). FAOSTAT Agriculture data. Food and Agriculture Organization of the United Nations. http://apps.fao.org/collections.

FAO. (2011). Ethiopia, yam production quantity for the year of 2008. Available online: http://www.factfish.com/statistic (Accessed: October, 2013)

Fradin, E. F. and Thomma, B. P. (2006). Physiology and molecular aspects of Verticillium wilt diseases caused by *Verticillium dahliae* and *Verticillium albo-atrum*. *Mol. Plant Pathol*. **7**: 71-86.

Gangadhara Naik, B., Nagaraja, R., Basavaraja, M. K. and Krishna Naik, R. (2004).Variability Studies of *Fusarium oxysporum* F. Sp. *Vanillae* Isolates. *Int. J. Sci. Nature* **1**: 12-16.

Hajieghrari, B., Torabi-Giglou, M., Mohammadi, M. R., and Davari, M. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *Afr. J. Biotechnol.* **7:** 967-972.

Hanson, L .E. (2000). Reduction of verticillium wilt symptoms in cotton following seed treatment with *Trichoderma virens. J. Cot. Sci.* **4**: 224-231.

Harish, S., Saravanakumar, D., Kamalakannan, A., Vivekananthan, R., Ebenezar, E. G. and Seetharaman, K. (2007). Phylloplane microorganisms as a potential biocontrol agent against *Helminthosporium oryzae* Breda de Hann, the inciting of rice brown spot, *Arch. Phytopathol. Plant Prot.* **40:**148 -157.

Harman, G., Howell, C., Viterbo, A, Chet, I. and Lorito, M. (2004). *Trichoderma* species opportunistic, virulent plant symbionts. *Nature Rev. Microbiol.* **2**: 43-56.

Hjeljord, L. and Tronsmo, A. (1998). *Trichoderma* and *Gliocladium* in biological control: an overview. **In**: *Trichoderma and Gliocladium Enzymes, Biological Control and Commercial Applications*, pp. 131-151, (Harma, G. E and Kubicek, C. P., eds). Taylor and Francis Ltd, London.

Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T. and Samiyappan, R. (2010). Effect of environmental conditions on growth of *Alternaria alternata* causing leaf blight of noni. *World J. Agri. Sci.* **6**: 171-177.

Huxley. (1992). Wikimedia Foundation, Inc., nonprofit organization. (Accessed: October, 2013). http://wikimediafoundation.org/

IITA. (2004). International Institute of Tropical Agric., Nigerian's Cassava Industry: Statistical Handbook. Ibadan, Nigeria, pp. 150.

Imran Khan, H. S., Saifulla, M., Mahesh, S. B. and Pallavi, M. S. (2011). Effect of different media and environmental conditions on the growth of *Fusarium oxysporum* F. Sp. *Ciceri* causing *Fusarium* wilt of chickpea. *Int. J. Sci. Nat.* **2**: 402-404.

Izekor, O. B. and Olumese, M. I, (2010). Determinants of yam production and profitability in Edo State, Nigeria. *Afr. J. General Agric.* **6**: 205-210.

Jabnoun-Khiareddine, H., Daami-Remadi, M., Barbara, D. J., and El Mahjoub, M. (2010). Morphological variability within and among *Verticillium* species collected in Tunisia. *Tunisian J. Plant Prot*. **5**: 19-38.

Johnson, D. (2003). Yams for food; *Dioscorea* spp. Paper presented at the 2002 Spring.

Junaid, J. M., Dar, N. A., Bhat, T. A., Bhat, A. H. and Bhat, M. A. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *Int. J. Modern Plant and Animal Sci.* **1**: 39-57.

Kamal, K. and Brian, G. (2006). Biological control of plant pathogens. The plant Health Instructor DOI: 10. 1094/ PHI-A-2006-1117-02.

Kay, D. E. (1987). *Crops and product digest No. 2 Root crops*. 2nd ed. London Tropical Development and Research Institue, Leiden, Netherlands. Pp. 380.

Kirk, P. M., Cannon, P. F., Minter, D. W. and Stalpers, J. A. (2008). *Dictionary of the Fungi. 10th ed*. Wallingford: CABI. Pp. 724.

Komesaroff, A., Black, V., Cable, V. and Sudhir, K. (2001). Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. *Climacteric* **4**: 144-150.

Kulkarni, S. P. (2006). Studies on *Fusarium oxysporum* Schlecht Fr f. sp. gladioli (Massey) sryd. and Hans causing wilt of gladiolus. Master thesis, University of Agricultural Sciences, Dharwad, India.

Lebot, V., Malapa, R. T., Molisade and Machad J. L. (2005). Physicochemical characterization of yam (*D. alata* L.) tubers from Vanuatu. Genetic Resources and Crop Evolution.

Leslie, J. F. and Summerell, B. A. (2006). The *Fusarium* Laboratory manual. Blackwell Publishing: Iowa, USA.

Li, Y. (2011). Biology characteristic determination of *Fusarium semitectum* in soybeans. *Advan. Biomed. Engin.***3**: 76-80.

Lilly, V. G. and Barnett, H. L. (1951). Physiology of Fungi. McGraw Hill Book Company Inc., New York, pp 464.

Link, H. F. (1809). Observationes in ordines plantarum naturals, Dissertatio I, Mag. Ges. Naturf. Freunde, Berlin 3, 3.

Manjula, K., Mwangi, M. and Bandyopadhyay, R. (2005). Potential of some bacteria and fungi as biocontrol agents of cassava and yam tuber rot pathogens under laboratory and green house conditions. *African Crop Science Conference Proceedings* **7**:1395-1400.

Mathew, M. A., Stephan, W., Hodeba, D. M., Kim, R. G. and Robert, A. (2003). Molecular taxonomic, epidemiological and population genetic approaches to understanding yam anthracnose disease. *Afr. J. Biotechnol*. **2**: 486-496.

Mathur, R.S., Barnett, H.L. and Lilly, V.G. (1950). Factors in-fluencing growth and sporulation of *C. lindemu-thianum* in culture. *Phytopathol.* **40**:104-114.

Mathur, S. B. and Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi, 2nd Edition. International Seed Testing Association.

Mignouna, H. D. and Dansi, A. (2003). Yam (*Dioscorea* spp) domestication by the Nago and Fon ethnic groups in Benin. *Genet. Resource Crop Evol*. **50**: 519-528.

Montealegre, J. R., Perez, L. M., Herrera, R, Silva, P. and Besoain, X. (2003). Selection of bioantagonisic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. *Environ. Biotechnol*. **6**: 1-9.

Morse, S., Acholo, M. N., McNamara, N. and Oliver, R. (2000). Control of storage insects as a means of limiting yam tuber fungal rots. *J. Stored Prod. Res.* **36**: 37-45.

Musa, Y. H., Onu, J., Vosanka, I. P. and Anonguku, I. (2011). Production efficiency of yam in Zing Local Government area of Taraba State, Nigeria. *J. Hort. Forest.* **3**: 372-378.

Muzac-Tucker, I., Asemota, H. N. and Ahmad, M. H. (1993). Biochemical composition and storage of Jamaican yams (*Dioscorea* sp). *J. Sci. Food Agric.* **62**: 219-224.

Naik, M. K., Hiremath, P. C. and Hegde, R. K. (1988). Physiological and nutritional studies on *Colletotrichum gloeosporioides*, a causal agent of anthracnose of beet levine. *Mysore J. Agric. Sci.* **22**:471-474.

Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. (1983): *Fusarium* species*:* an illustrated manual for identification. The Pennsylvania State Univ., Press, University Park.

Nene, Y. L. and Thapliyal, P. N. (1993). *Fungicides in Plant Disease Control*. Oxford and IBH Publishing Co, New Delhi, India. pp 579.

Nisa, T., Wani, A. H., Bhat, M.Y., Pala, S.A. and Mir, R. A. (2011). *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *Journal of Biopesticides* **4**: 53-56.

Noon, R. A. and Calhoun (1979). Market and storage of yams imported to United Kingdom. *Phytopath.* Z. **94**: 289-302.

Odior, A. O. and Oyawale, F. A. (2012). Analysis of production capacity of a yam flour producing firm using a mathematical model. *J. Eng. Appl. Sci.* **7**:820-824.

Ogali, E. L., Opadokun, J. S. and Okobi, A. O. (1991). Effects of lime and local gin on postharvest rot of yams. *Trop. Sci.* **31**: 365-370.

Ogbuagu, M. N. (2008). Nutritive and anti - nutritive composition of the wild (inedible) species of *Dioscorea bulbifera* (potato yam) and *Dioscorea dumetorum* (bitter yam). *The Pacific J. Sci. Tech.* **9**: 203-207.

Ogundana, S. K., Naqui, S. H. and Ekundayo, J. A. (1970). Fungi associated with soft rot of yam (*Dioscorea* spp) in Nigeria. *Trans. Br. Mycol. Soc.* **54**: 445-451.

Okafor, N. (1966). Microbial rotting of stored yams (Dioscorea spp) in Nigeria. *Exp. Agric*. **2**:179-182.

Oke, O. L. (1990). Roots, Tubers, Plantain and Bananas in Human Nutrition, FAO Food and Nutrition Series No. 24.

Okigbo, R. N. (2003). Fungi associated with peels of post harvest yams in storage. *Global J. Pure Appl. Sci.* **9**: 19-23.

Okigbo, R. N. and Emoghene, A. O. (2004). Antifungal activity of leaf extracts of some plant species on *Mycosphararella fijiensis* Morelet, the causal organism of black sigatoka disease in banana *(Musa acuminata*). *Sci. J.* **4**: 20-31.

Okigbo, R. N. and Ikediugwu, F. E. O. (2000). Studies on biological control of postharvest rot of yam with *Trichoderma viride*. *J. Phytopathol*. **148**: 351-355.

Okigbo, R. N. and Ikediugwu, F. E. O. (2002). Evaluation of water losses in different regions of yam (*Dioscorea* spp.) tuber in storage. *Nig. J. Exp. Appl. Bio.* **3**: 320.

Okigbo, R. N. and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam rot. *Afr. J. Biotechnol.* **5**: 727-731.

Okigbo, R. N. and Osuinde, M. I. (2003). Fungal leaf spot diseases of mango (*Mangifera indica*) in South Eastern Nigeria and biological control with *Bacillus subtili*. *Plant Prot. Sci.* **39**: 54-69.

Olaoye, J. O. and Oyewole, S. N. (2012). Optimization of some "poundo" yam production parameters. *Agric. Eng. Int. CIGR J.* **14**: Manuscript No. 2063.

Omer, K. M. and Shahzad, S. (2007). Screening of *Trichoderma* species for tolerance to fungicides. *Pak. J. Bot*. **39**: 945-951.

Ommati, F. and Zaker, M. (2012). Evaluation of some *Trichoderma* isolates for biological control of potato wilt disease (*Fusarium oxysporum*) under laboratory and greenhouse conditions. *J. Crop Prot.* **1**:279-286.

Opara, L. U. (1999). Yam storage. **In**: *CIGR Handbook of Agricultural Engineering Volume IV Agro Processing.* pp. 182-214, (Bakker-Arkema *et al*., eds). The American Society of Agricultural Engineers, St. Joseph, MI.

Opara, L. U. (2001). Onions: Post-harvest operations in developing countries. INPhO Compendium, FAO, Rome (in press).

Osagie, A. U. (1992). The yam tuber in storage. *Postharvest Res. Unit* **11**: 174-181.

Osuinde, M. I., Egogo, H. and Okigbo, R. N. (2002). Effects of isolate of *Trichoderma* spp on *Fusarium oxysporum* f.s.p lycopersici in *in vitro*. *Nigerian J. Microbiol.* **15**: 125-130.

Otsuka, H., Tamari, K. and Agaraswara, N. (1957). Biochemical classification of *Piricularia oryzae* cav. *J. Agric. Chem. Soc. Japan* **31**:791-798.

Pandey, A. (2010). Antagonism of two *Trichoderma* species against *Alternaria alternata* on *Capsicum frutescens*. *J. Exp. Sci.* **1**: 18-19.

Papavizas, G. C. (1985). *Trichoderma* and *Glicladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathol.* **23**: 23-54.

Pegg, G. F. and Brady, B. L. (2002). *Verticillium* Wilts. CABI Publishing, New York. Pp. 167.

Ramjegathesh, R. and Ebenezer, E. G. (2012). Morphological and physiological characters of *A. alternata* causing leaf blight disease of onion. *Int. J. Plant Pathol*. **3**: 34-44.

Ramteke, P. K. and Kamble, S. S. (2011). Physiological studies in *F. solani* causing rhizome rot of ginger (*Zingiber officinale* Rosc.). *Int. Quart. J. Life Sci.* **6**: 195-197.

Rita, N. and Tricita, H. Q. (2004). Soil mycoflora of black pepper rhizosphere in the Philippines and their *in vitro* antagonism against *phytophthora capsici* L. *Indo. J. Agric. Sci*. **5**: 1-10.

Rocco, A. and Perez, L. M. (2001). *In vitro* biocontrol activity of *Trichoderma harzianum* on *Alternaria alternata* in the presence of growth regulators. *Plant Biotechnol*. **4**: 68-72.

Rutherford, M. A. (2006). Current knowledge of coffee wilt disease, a major constraint to coffee production in Africa. *Phytopathol.* **96**: 663-666.

Sahi, I. Y. and Khalid, A. N. (2007). *In vitro* biological control of *Fusarium oxysporum* causing wilt in *Capsicum annuum*. *Mycopath* **5**: 85-88.

Sangdee, A., Sachan, S. and Khankhum, S. (2011). Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chilli in the Northeast of Thailand. *Afr. J. Microbiol. Res*. **5**: 4368-4372.

Sanni, L. O., Oyewole, O. B., Adebowale, A. A. and Adebayo, K. (2003). Current trends in the utilization of roots and tubers for sustainable development, 2nd international workshop, *Food based approaches for a healthy nutrition,* Ouagadougou, 23-28.

Sempere, F. and Santamarina, M. P. (2007). *In vitro* biocontrol analysis of *Alternaria alternata* (Fr.) keissler under different environmental conditions. *Mycopath.* **163**: 183-190.

Serge, T. and Agbor-Egbe, T. (1996). Biochemical changes occurring during growth and storage of two yam species. *Int. J. Food Sci. Nutri.* **47**: 93-102.

Shanthakumari, S., Mohan, V. and Britto, J. (2008). Nutritional evaluation and elimination of toxic principles in wild yam (*Dioscorea spp*.). *Tropical and Subtropical Agroecosystems* **8**: 319-225.

Shehu, J. F., Iyortyer, J. T., Mshelia, S. I. and Jongur, A. A. U. (2010). Determinants of yam production and technical efficiency among yam farmers in Benue State, Nigeria. *J. Soc. Sci.* **24**: 143-148.

Silimela, M. and Korsten, L. (2001). Alternative methods for preventing pre and post-harvest diseases and sunburn on mango fruits. S.A. Mango Growers‟ Assoc. *Yearbook* **21**: 39-43.

Simmons, E. G. (2007). *Alternaria.* An identification manual. CBS Biodiversity Series 6. CBS Fungal Biodiversity Centre, Utrecht, the Netherlands. Pp. 306.

Smith, S. N. (2007). An over view of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil borne pathogenic forms. *Plant Path. Bulletin* **16**: 97-120.

Srinivas, P. and Ramakrishnan, G. (2002). Use of native microorganisms and commonly recommended fungicides in integrated management of rice seed borne pathogens. *Annu. Plant Prot. Sci.* **10**: 260-264.

Suleiman, M. N. (2010). Fungitoxic activity of neem and pawpaw leaves extracts on *Alternaria solani*, causal organism of yam rots. *Adv. Environ. Biol.* **4**: 159-161.

Sunita, S. C. (1999). Fungal and Bacterial Disease of Bulbous Ornamentals, **In:** *Diseases of Horticultural Crops-Vegetables, Ornamentals and Mushrooms,* pp. 501-531, (Verma L.R. and Sharma R. C., Eds). Indus Publishing Co., New Delhi.

Suprapta, D. N. (2012). Potential of microbial antagonists as biocontrol agents against plant fungal pathogens . *J. ISSAAS* **18**: 1-8.

Sweetmore, A., Simons, S. A. and Kenward, M. (1994). Comparison of disease progress curves for yam anthracnose (*Colletotrichum gloeosporioides*). *Plant Path.* **43**: 206-215.

Thangavelu, R., Velazhahan, R. and Sathiamorthy, S. (2003). Biocontrol of *Fusarium* wilt diseases. **In:** *2nd International symposium of Fusarium wilt on banana,* Pp.34, National Research center for banana. Triruchirapalli, Tami Nadu, India.

Thomas, O. (2012). Effects of processing methods on nutrient retention and contribution of white yam (*Dioscorea rotundata*) products to nutritional intake of Nigerians. *Afric. J. Food Sci.* **6**: 163-167.

Ulbricht, C., Basch, E. and Sollars, D. (2003). Wild yam. *J. Herbal Pharmacotherap.* **3**: 77-91.

Umeta, M., West, C. E. and Fufa, H. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *J. Food Composition and Analysis* **18**: 803-817.

Van Bruggen A.H.C. (1984). Sweet potato stem blight caused by *Alternaria* spp a new disease in Ethiopia. *Netherlands Journal of Plant Pathogen* **1**: 155-164.

Vloutoglou, I. and Kalogerakis, S. N. (2000). Effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria solani*) and on shedding of leaves in tomato plants. *Plant Path*. **49**: 339-345.

Walsh, S. (2003). Plant Based Nutrition and Health. Vegan Society Ltd. pp 240.

Woudenberg, J. H. C., Groenewald, J. Z., Binder, M. and Crous, P. W. (2013). *Alternaria* redefined. *Studies in Mycology* **75**: 171-212.

Yonas Urbanos (2010). Characterization of antagonistic *Trichoderma* species against Coffee Wilt Disease (*Fusarium xylarioides).* Master thesis, University of Addis Ababa, Ethiopia.

Yukie, K. (2008). Aminoglycosides and Syringomycin E as Fungicides against *Fusarium graminearum* in head blight disease. Master thesis, Utah State University, Utah.

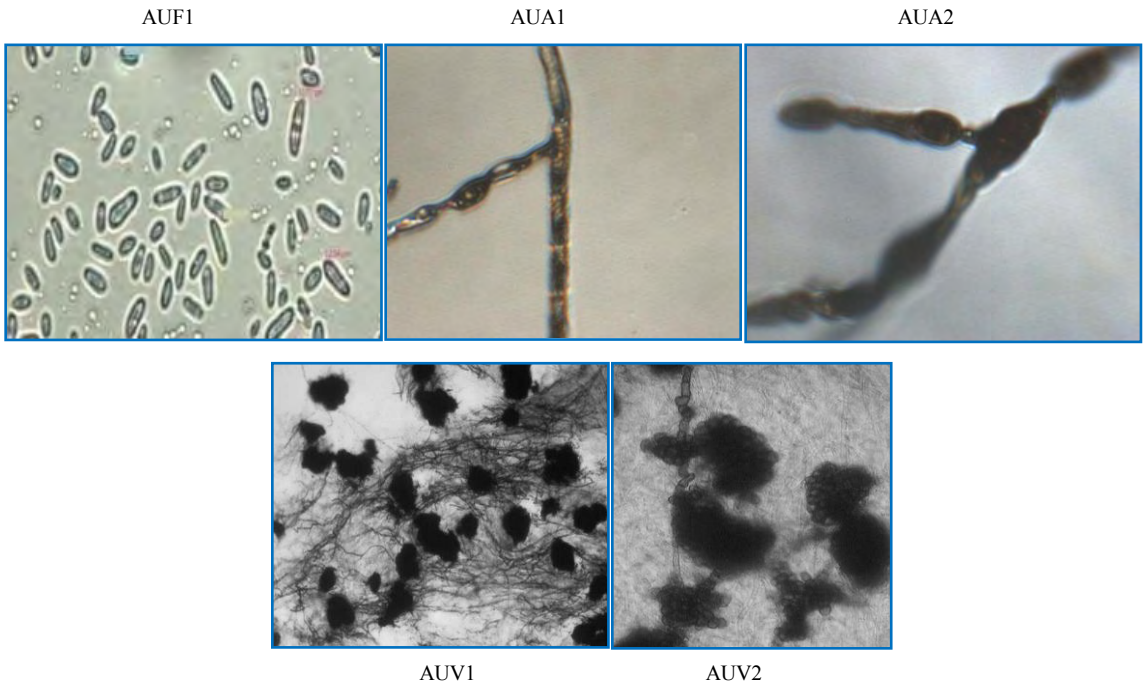
APPENDICES

Appendix 1 Cultural characteristics of fungal isolates isolated and identified from yam

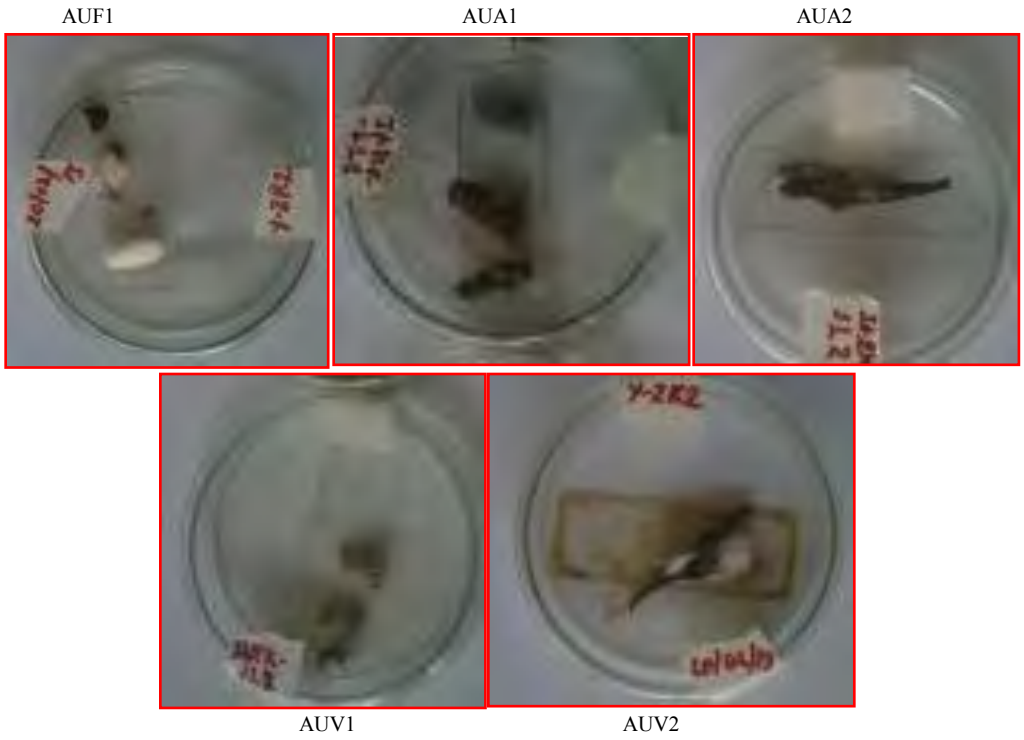
AUF1 AUA1 AUA2

AUV1 AUV2

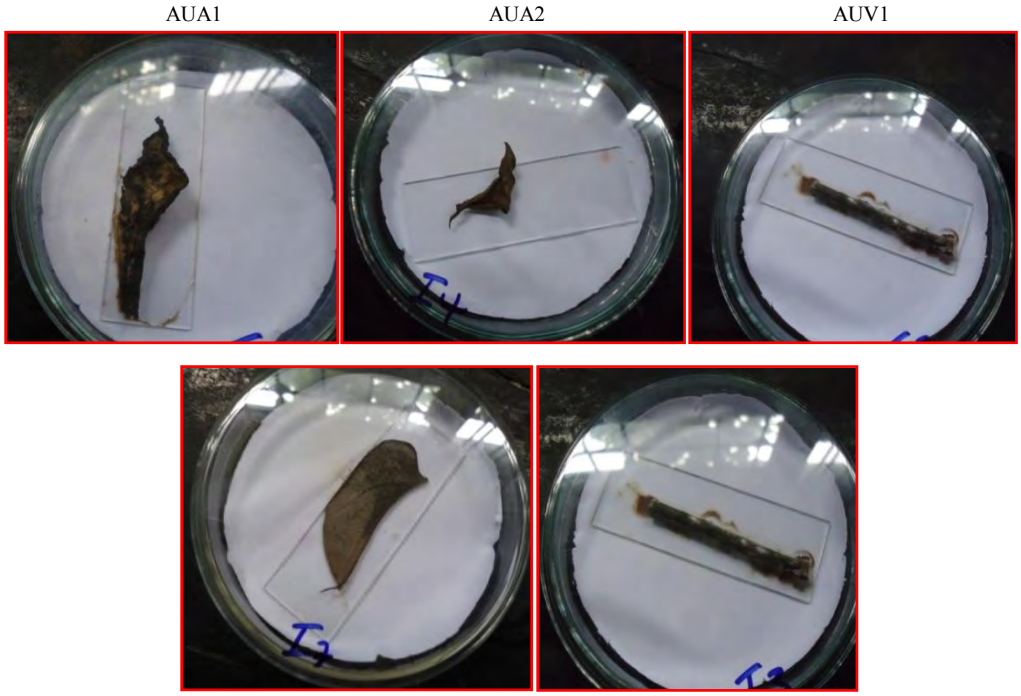
Appendix 2 Spore morphology of genera of fungal isolates attachment to mycelia when slide culture was observed under microscope (40×)



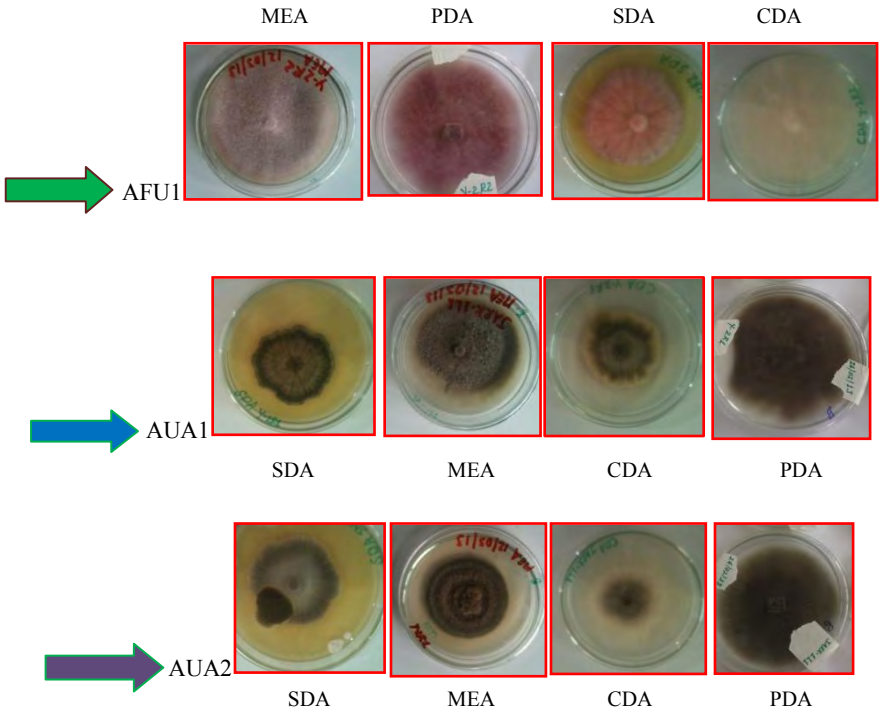
Appendix 3 *In vitro* pathogenicity test of fungal isolates on yam cultivar after 20 days of incubation at 25^{o}C



Controls

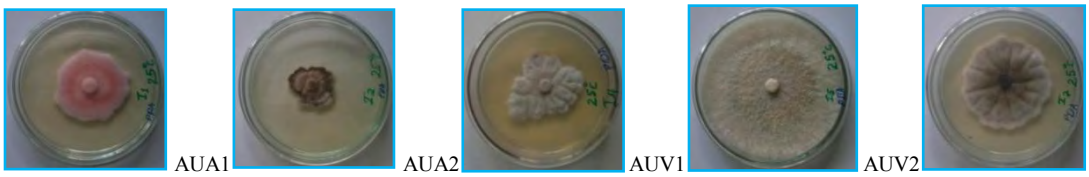


Appendix 4 The effect of different solid media on the mycelial growth of fungal isolates after ten days of incubation at 25^{o}C

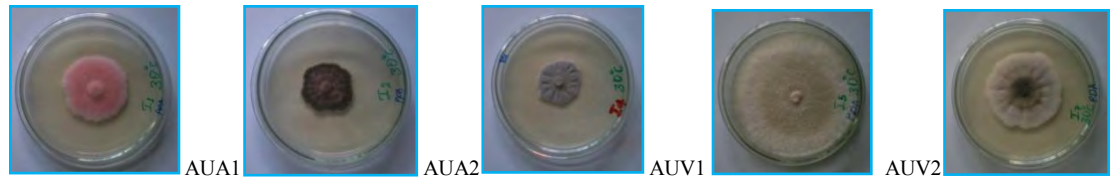


Appendix 5 The effect of different temperatures on the mycelial growth of fungal pathogens after eight days of incubation

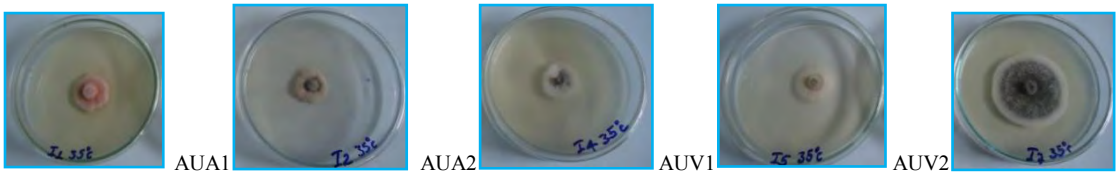
Temperature @ 25°C



Temperature @ 30°C

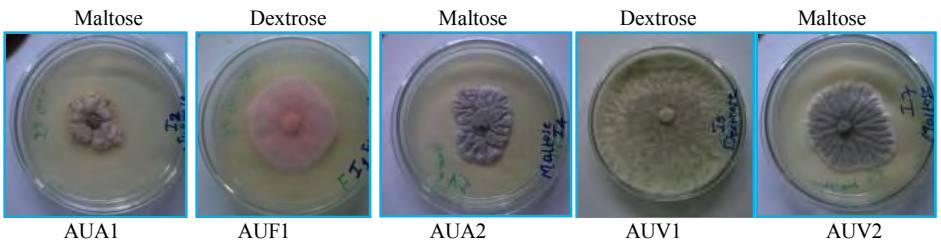


Temperature @ 35° C

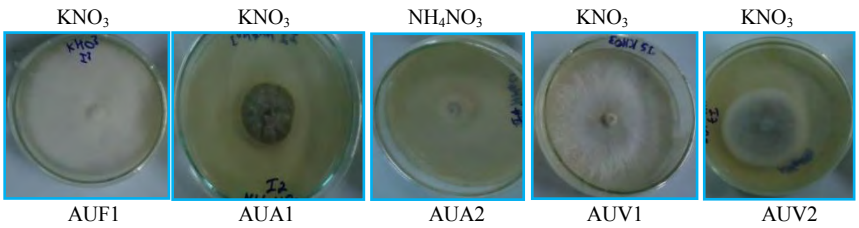


Appendix 6 Fungal isolates grown in PDB to measure their dry mycelial weight at different pH levels after 15 days of incubation

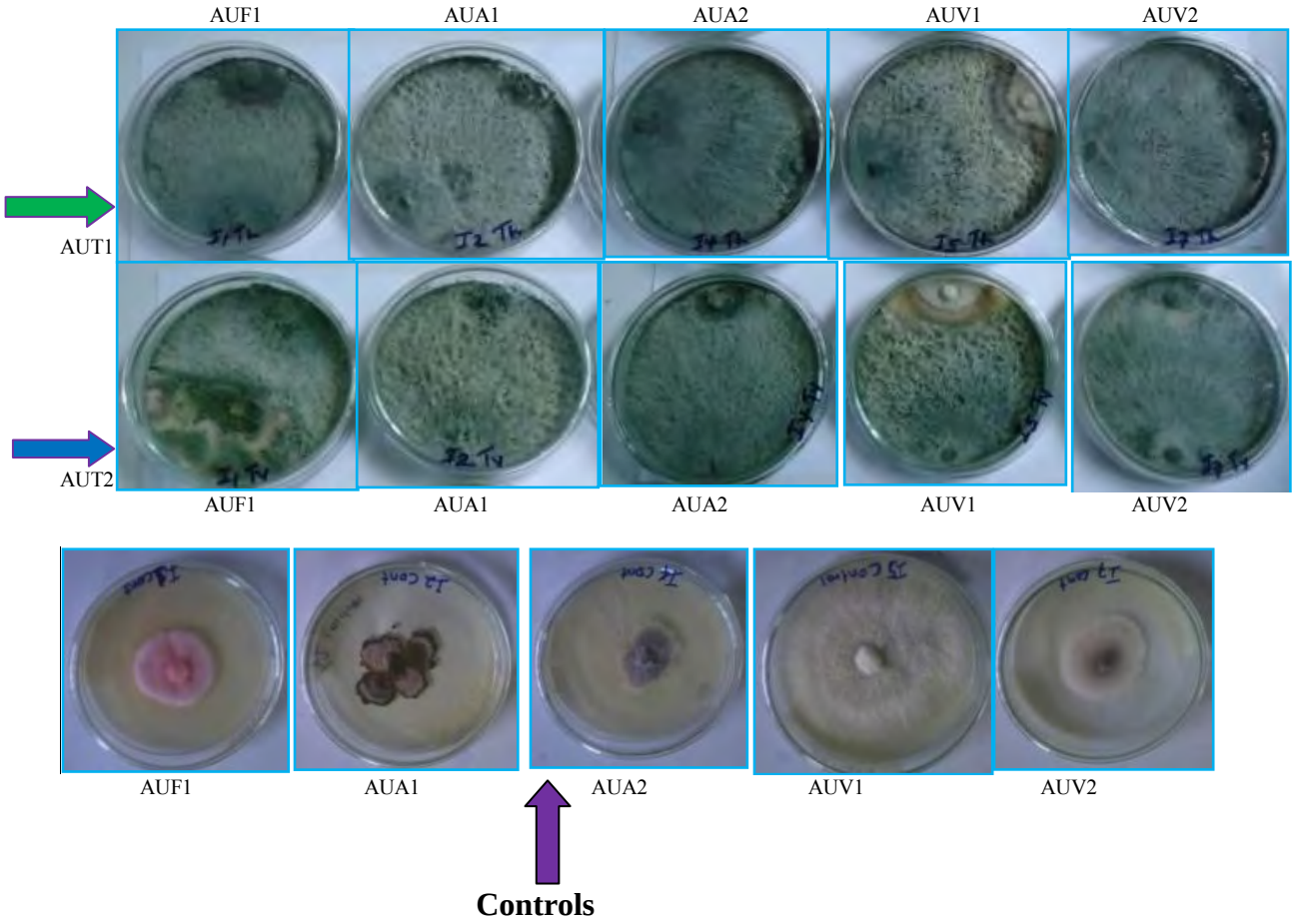
Appendix 7 The different carbon sources on which the maximum mycelial growth of each fungal isolates were attained after ten days of incubation 25°C



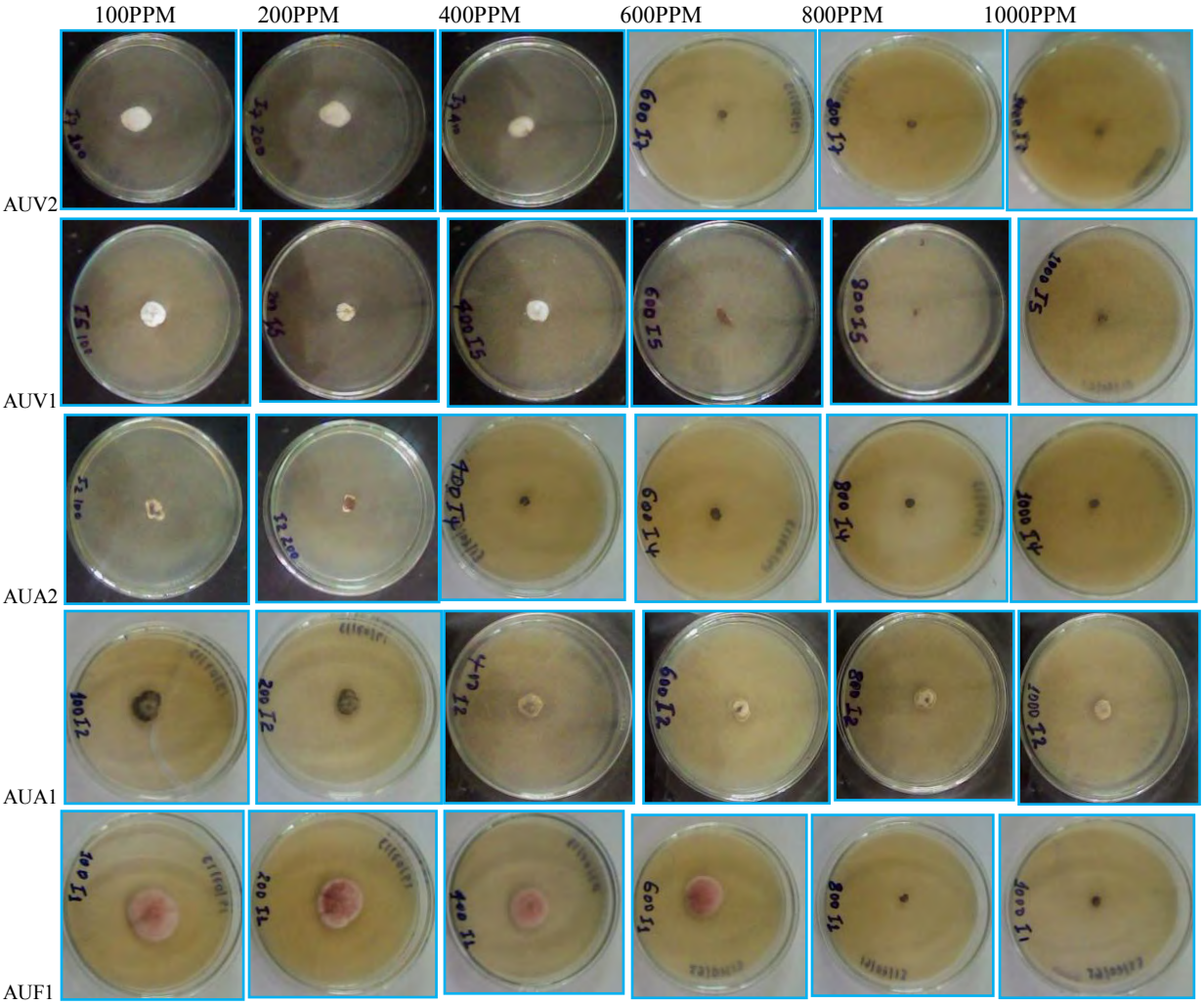
Appendix 8 The different nitrogen sources on which the maximum mycelial growth of each fungal isolates were attained after ten days of incubation at 2 ^{o}C



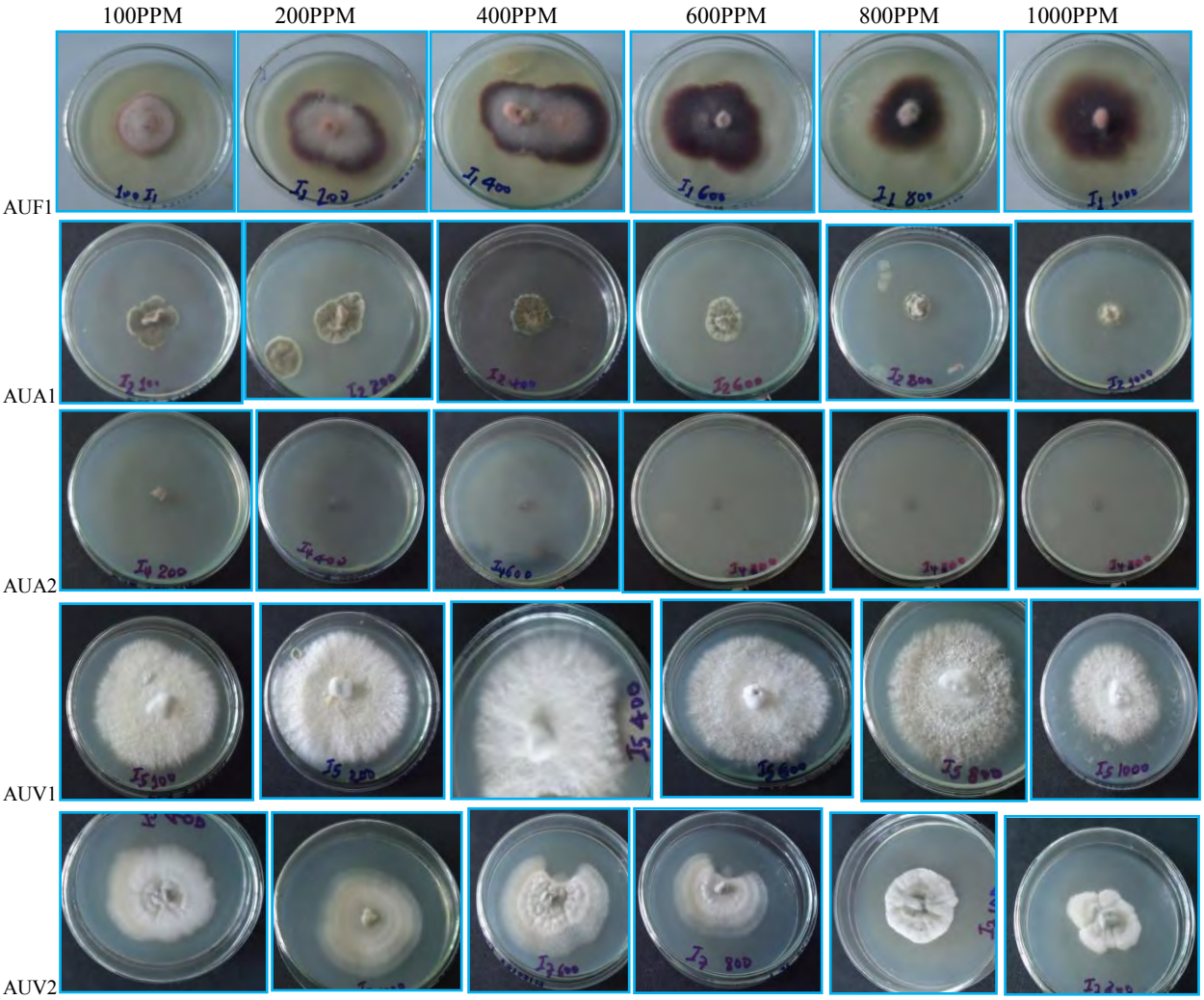
Appendix 9 *In vitro* evaluation *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) against fungal isolates after seven days of incubation

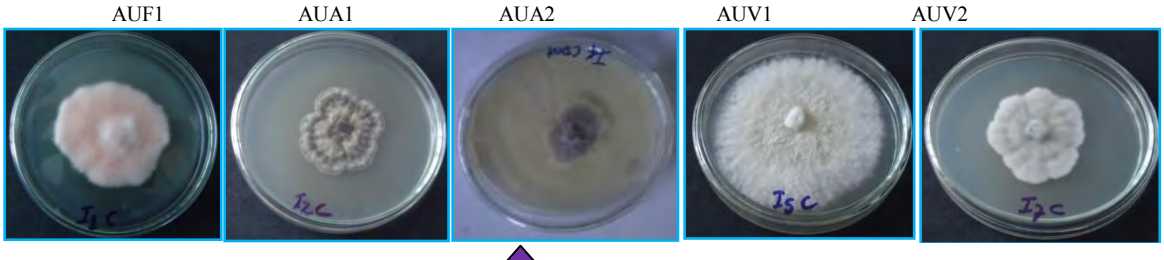


Appendix 10 *In vitro* evaluation of sancozeb, 80% WP at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm concentrations on mycelial growth of fungal isolates after ten days of incubation at 25 ^{o}C



Appendix 11 *In vitro* evaluation of curzate, 43.95% WP at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm concentrations on mycelial growth of fungal isolates after ten days of incubation at 25^{o}C





Controls stand for both sancozeb and curzate fungicides