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Evaluation of *Trichoderma* species against *Fusarium* Wilt and *Alternaria* Leaf Blight of Sesame (*Sesamum indicum* L.) Under *in vitro* condition

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of APPENDECES

ACRONYMS AND LIST OF ABBREVIATIONS

AAU	Addis Ababa University
ALS	Alternaria leaf spot
AUT	Addis Ababa University *Trichoderma* isolates
BCA	Biological control agent
CSA	Central Statistical Agency
CWDE	Cell-wall- degrading enzymes
CZI	Clear zone of inhibition
FOS	*Fusarium oxysporum* f. species *sesami*
ISR	Induced systemic resistance
ITSR RNA	Internal transcribed spacer region of the ribosomal RNA gene
LDL	Low density lipoprotein
MLOs	Mycoplasma–like organisms
PIRG	Percent inhibition of radial growth
SAR	Systemic acquired resistance

Evaluation of *Trichoderma* species against *Fusarium* Wilt and *Alternaria* Leaf Blight of Sesame (*Sesamum indicum* L.) Under *in vitro* conditions

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ABSTRACT: The present study was aimed to evaluate and characterize the potential biocontrol agents of *Trichoderma* isolates against two pathogenic fungi viz., Alternaria isolates (AUA1) and *Fusarium* isolates (AUF5) under *in vitro* condition. Infected leaves, seeds and soil samples of sesame were collected from different habitats of the Wolkait district for the isolation of fungal pathogens. The pathogenicity test of these isolates was confirmed on detached leaves of sesame plants resulting in typical symptoms of leaf blight and wilt disease. In this study, the *in vitro* potential of 7 *Trichoderma* isolates were evaluated against two isolates of foliar and soil borne phytopathogenic fungi in dual culture techniques and through production of volatile and non-volatile inhibitors. The effect of pH and temperature on the mycelia growth and spore yield of *Trichoderma* isolates were determined PDB medium. While, the optimum temperature for mycelial growth and the maximum spore yield was produced at 25^{O}C. Among the seven *Trichoderma* isolates, AUT-12 (93 %), AUT-158 (91.6 %) and AUT-97 (90.8%), exhibited a significant ($P< 0.05$) enhancement of germination percentage in sesame seeds under *in vitro* conditions, while the control treated significantly ($p<0.05$) decreased these values (55 %). *In vitro* confrontation analysis revealed that all the *Trichoderma* isolates were highly antagonistic and AUT-131 isolate displayed over 75% inhibition of mycelial growth against both test pathogens under dual culture. Except isolates AUT-32 and AUT-158, all the other *Trichoderma* isolates showed consistent results in dual culture test, volatile and non-volatile activity against any of the two pathogens tested. Under in-vitro dual culture test, the experimental results showed that all the isolates of *Trichoderma* were able to inhibit the growth of both test pathogens at rates ranging from 66.29% to 79.49% after 6 days of incubation. The highest mean inhibitory effect on the growth of the test pathogens were achieved by AUT-131 isolate (79.49%) against AUF5 and AUT-97 isolate (76.42%) against AUA1 while AUT-33 isolate showed the lowest mean inhibitory effect restricting it almost completely in plates compared to the control consisting of any of the two test pathogen growing alone. The highest (90.12%) non –volatile inhibition effect was exhibited by both AUT-11 and AUT-12 against AUF5 isolate, while the lowest (16.38%) inhibition was obtained by AUT-32 isolate. Thus, the use of novel isolates of *Trichoderma* with efficient antagonistic capacity against *Alternaria* (AUA-1) and *Fusarium* (AUF5) isolates is a promising alternative strategy to fungicides for sesame disease management.

Keyword/phrases: Antagonism**,** Biocontrol, Fungal pathogens, non-volatile inhibitors and Volatile metabolites.

1. INTRODUCTION

1.1 Background

Sesame (*Sesamum indicum (L.*)), belongs to family *Pedaliaceae* is considered as one of the most ancient oilseed crops known to mankind. It is also known as Benn seed in Africa and sim-sim in East Africa (Ashri, 1998; Nafe *et al.*, 2010). The genus has many species, and most of them are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. *S. indicum,* the cultivated type originated in India (Sheahan, 2014). It is extensively cultivated in the tropics and the temperate zone of the world (Naderikharaji *et al.*, 2008; Zerihun, 2012). India, China, Burma, Sudan and Mexico are the largest producers (Ijlal *et al.*, 2011). According to Raney *et al.* (2011), the global cultivated areas of sesame are about 7.4 million hectares producing about 3.4million metric tons, which make it the fifth most important oilseed crop on an area basis worldwide. Raney *et al.* (2011) have reported that, Sudan grows about 20.12% (1.48 million hectares) of the world cultivated area, and contributes about 9.24 %(0.32 million metric tons) of the total production. Sesame is the second largest source of foreign exchange earner after coffee in Ethiopia (Wijnands *et al.*, 2011) as it is the major oil seed in terms of exports in Ethiopia, accounting for over 90% of the values of oilseed exports (Kindie, 2007).

Sesame is considered to have both nutritional and medicinal values. It is grown mainly for its seeds that contain approximately 35- 60% oil (El Khier *et al.*, 2008) and 25% protein (Weigle *et al.*, 2005).The sesame seed has long shelf life due to the presence of lignans, which have a remarkable antioxidant function. The seed is highly rich in quality proteins and essential amino acids. The seed is also a rich source of linoleic acid, vitamin E, A, B1 and B2 and minerals including calcium and phosphorus. It is grown primarily for its nutritious seeds that are rich in linoleic acid, protein, and calcium as well as vitamin E and small quantity of vitamins A, B1and B2.Nearly 70% of the world's sesame seed is processed for oil and meal, while the remainder is channeled to food and confectionery industries (Morris, 2002). Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier *et al.*, 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavor or garnish foods, they were primarily used for oil and wine.

Among the major factors responsible for lower yields, diseases play an important role. Sesame is also affected by many biotic and abiotic stresses. Among the biotic agents, fungi cause major diseases, followed by bacteria, viruses and nematodes.

Among these diseases, at present leaf spot / blight caused by *Alternaria sesami* and *Fusarium* wilt caused by *Fusarium oxysporum* (the focus of this study) are widespread and have continued to be the major constraints in the production and productivity of sesame in Ethiopia. *Alternaria* leaf spot (ALS) of sesame caused by the seed-borne fungus *Alternaria sesami* (Kaw.) Mohanty and Behera are of worldwide importance (Leppik and Sowell, 1964). *Alternaria*s pecies are spread from field to field and from one geographical area to another by several means, including wind-borne spores and adherence of soil to seedlings, farm equipment, or animals (Ojiambo *et al.*, 2003). In some pathosystems, viable spores pass through the digestive tract of ruminants fed on diseased plant tissue. This may be an important means of spread of the pathogen from infested to non-infested areas within a field, or from infested fields to non-infested fields (Melouk *et al.*, 1989). On the other hand, *Fusarium* wilts caused by *Fusarium oxysporum f. spp. Sesami* (FOS) is a devastating disease infecting sesame crop right from seedling to maturity resulting in yield losses in varying degrees depending on the severity of infection. It has been reported as a most important soil borne fungal disease in which the water-conducting (xylem) vessels become blocked, so that the plant wilts and often dies (Zhang *et al.*, 2019).

The use of chemicals to control plant disease is an effective and successful approach. However, the large-scale use of chemical pesticides has resulted in problems including safety risks, environment pollution, and biodiversity loss and chemical resistance development by pathogens. Hence, it is quite prudent to develop a more effective and eco-friendly strategy, instead of chemical pesticides for controlling plant diseases. Biological control, such as using antagonistic microorganisms offers an attractive protection of crops from diseases unlike chemicals (Pedlowski *et al.*, 2012). In the literature, the use of biological control agents (BCAs) has been documented as a potential alternative to control *Fusarium* species (Tian *et al.*, 2016) and *Alternaria* species (Rajput *et al.*, 2013; Ryu *et al.*, 2006). In recent years, *Bacillus* (Comby *et al.*, 2017), *Pseudomonas* (Alimi *et al*., 2012; Hu *et al*., 2014; Müller *et al*., 2016; Wang *et al*., 2015), *Trichoderma* (Hasan *et al*., 2012; Matarese *et al*., 2012;

Schöneberg *et al*., 2015) and *Cryptococcus* (Schisler *et al*., 2014) have been the most commonly investigated microorganisms for the control of *Fusarium* species tested under *in vitro* condition (Haran *et al.*, 1996).

Fungal biological control agents can be ideal control strategies for fungal pathogens as they can be self-propagating, confer resistance via multiple strategies, have low toxicity, or otherwise beneficial to the plants, and can be used in accordance with organic farming practices (Bunbury-Blanchette and Walker, 2019). Ideal agents are common and abundant in most soil types, so increasing their numbers does not cause overt detrimental changes to the soil micro biota or biochemistry. The fungal genus *Trichoderma* contains species that are well documented as biocontrol agents of many crop pathogens, including *Fusarium* species (Degenkolb *et al.*, 2015; Reino *et al.*, 2008). Depending on species (*Trichoderma* sp., pathogen, and host plant), members of the genus *Trichoderma* may employ one or more modes of antagonism such as the production of toxins (e.g. antifungals, chitinase), mycoparasitism (physical disruption of pathogen hyphal growth: coiling, penetration, dissolution of cytoplasm), induction of a host defense response, or success in monopolizing rhizosphere nutrients and space (Gajera *et al.*, 2013; Howell, 2003; Raaijmakers *et al.*, 2009).

In recent years, the filamentous fungi *Trichoderma* species have attracted much research attention because they are effective biocontrol agent against a wide range of plant pathogens (Dubey *et al.*, 2007; Harman *et al.*, 2004; Hermosa *et al.*, 2012; Waghunde *et al.*, 2016). *Trichoderma* species act upon pathogens either directly by mycoparasitism, producing cell wall-lytic enzyme, and antimicrobial. Compounds, or indirect effects on plant pathogens by competing for nutrients and space, are modifying the environment conditions, promoting plant growth, an indirect systemic defense response to the pathogen of plants as well as increasing plant nutrient availability (Benítez *et al.*, 2004; Ruocco *et al.*, 2015).

In general, commercial preparations of *Trichoderma* species for biological control consist of bulk-produced conidia, which are the asexual reproductive units of this fungus. Bulk production of conidia typically relies on the manipulation of nutrients and substrates to promote conidiation. The optimum pH and temperature are the main environmental factors influencing conidiation in *Trichoderma* species. *Trichoderma* species are of great importance

as biocontrol agents that have better stress tolerance levels than the plant pathogens against which they are going to be used for biological control.

Trichoderma species serve as control agents of *Fusarium* and *Alternaria* species. There is strong support that one or more *Trichoderma* species would be effective antagonists of *Fusarium* wilt and leaf blight of sesame (Jat and Agalave, 2013). We evaluated the biocontrol potential of seven *Trichoderma* species isolated from different local agricultural soils against the causative agent of *Fusarium* wilt and leaf blight of sesame. *Trichoderma* isolates were tested for antagonistic activity toward both fungal pathogens of sesame under *in vitro* condition.

1.2 Statement of the problem

Pathogenic fungi are the most common and economically important foliar and soil borne diseases of sesame. Presently, *Alternaria* leaf spot (ALS) is controlled primarily through foliar application of chemicals (Aktar *et al.*, 2009). However, this management option is not economical for resource-limited small-scale farmers who are the major producers of sesame in Ethiopia. Availability of healthy seed that have tolerable levels of *A. sesame* (Ojiambo *et al.*, 1999) and exclusion of the disease from non-infested sesame growing areas are some of the practical management options for controlling ALS. In addition, *Fusarium* wilt of sesame caused by *Fusarium oxysporum* remains a challenging task in terms of management in Ethiopia (Gelalcha, 2009). Application of fungicides, crop rotation with non-hosts of the fungus and disinfestations of the soil to control these diseases is not economical and labour intensive. Besides, chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition, pesticides also kill various beneficial organisms. Toxins persist in soil can contaminate the whole environment. Prospects of biological control of soil-borne plant pathogens using the most promising biocontrol agent, the genus *Trichoderma* has been taken and its potential was evaluated to control fungal diseases of sesame crop under *in vitro* condition. Therefore, the main purpose of this study was to evaluate the interaction between the antagonists *Trichoderma* isolates against sesame fungal pathogen isolates.

1.3 Objectives

1.3.1 General objective

- The general objective of this study was to isolate, identify, characterize and evaluate *Trichoderma* species against *Fusarium* wilt and *Alternaria* leaf blight of sesame (*Sesame indicum* L.) under *in vitro* conditions.

1.3.2 Specific objectives

- To isolate, identify and characterize fungal pathogens of sesame.
- To determine the effects of *Trichoderma isolates* using the dual culture test, volatile and non-volatile compounds on *Fusarium* isolates (AUF5) and *Alternaria* isolates (AUA1).
- To examine the effect of pH and temperature on the mycelia growth and spore yield of *Trichoderma isolates* in batch culture.

2. LITERATURE REVIEW

2.1 Sesame crop

Sesame (*Sesamum indicum L.)* is one of the most important oilseeds crops worldwide, and has been cultivated in Africa for use as a traditional health food. Sesame seeds are used in making of tahini (sesame butter) and for the preparation of rolls, crackers, cakes and pastry products in commercial bakeries. There are numerous varieties and ecotypes of sesame adapted to various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information (Ashri, 1998). Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (Brown and Funk, 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavor or garnish foods, they were primarily used for oil and wine. Sesame seed is harvested when about 50% of capsules turn yellow in color from green. Other indications of the optimum time for harvesting (physiological ripeness) include; lowest capsules turning brown and beginning to pop open, stem turning yellow, leaves begin to fall off, end of blossoming, leaves turning yellow (Kindie, 2007).

2.2. Origin and Distribution of sesame

Discussion continues about the exact origin of sesame. It is often asserted that sesame has its origin in Africa and spread early through western Asia, China and Japan, which themselves became secondary centers of diversity. With the exception of *Sesamum prostrayum Retz,* all the wild *Sesamum species* are found in Africa. This variability and the importance of the sesame in the economies of several African countries could further justify the African continent to be the ultimate center of origin. However, Bedigian, (2004) demonstrated that the crop was first domesticated in India, citing morphological and cytogenetic affinities between domesticated sesame and the south India native *S. mulayanum* Nair, as well as archeological evidence that it was cultivated at Harappa in the Indus valley between 2250 and 1750 BC. All these assertions make it difficult to say with certainty the exact origin of the crop. Bedigian (2004) observed that the relatively low productivity sesame ranks only ninth among the top thirteen oilseed crop, which 90% of the world production of edible oil.

2.3 Taxonomy, morphology and ecology of sesame

Sesame (*Sesamum indicum (L.*)), belongs to order *tubiflera* and family *Pedaliaceae* which contains 60 species organized into 16 genera (Nafe *et al.*, 2010). It is known by common names Beni, benne and Beni seed (Sheahan, 2014). It is an annual self-pollinating plant with an erect, pubescent, branching stems (Morris, 2002). Sesame crop contains 13 pair of the chromosome (Chromosome No. 2N = 26) ((Nafe *et al.*, 2010).

It is a broad-leaved plant that grows about 155 to 185 cm tall, with height dependent on the variety and growing conditions. Large, white, bell-shaped flowers, each about 2.5 – 5 cm long, appear from the leaf axis on the lower stem, then gradually appear up the stem over a period of weeks as the stem keeps elongating (Sheahan, 2014). Depending on the variety, either one or three seed capsules will develop at each leaf Axil. Seed capsules are 2.5 to 3.8 cm long, with 8 rows of seeds in each capsule. Some varieties are branched, while others are un-branched. It is propagated by seed and takes about four months for the seeds to ripen fully. The leaves vary from ovate to lanceolate and are hairy on both sides (Anilakumar *et al.*, 2010). There are two types of sesame with regard to pod opening behavior, shattering and non-shattering (dehiscent).

Almost all sesame cultivars in Ethiopia are shattering type, which open by cracking of pods from top to bottom and releasing all seeds to fall on the ground. The dehiscent varieties have effective seed retention mechanisms, which makes them suitable for machine harvesting or even for traditional but late harvesting (Terefe *et al.*, 2012). Sesame (*Sesamum indicum* L.) is grown in areas with annual rainfall of 625-1100mm and temperature of $>27^{O}_{C}$. It is often grown where cotton can grow, under conditions few other crops can survive, requiring very few inputs (Sheahan, 2014). It is considered as a drought tolerant crop and is therefore mainly grown as a dry land crop (Ali and Jan, 2014), but not to water logging and excessive rainfall. It is well adapted to a wide range of soils, but requires deep, well-drained, fertile sandy loams (Terefe *et al.*, 2012). Clay soils are more prone to water logging. Sesame will not withstand water over the stem because it limits oxygen presence to the roots and suffocates the plants. Sesame prefers slightly acid to alkaline soils (pH 5-8) with moderate fertility (Sheahan, 2014 and Zerihun, 2012). In Ethiopia, sesame grows well in the semi-arid areas of Amhara, Tigray, Benishangul Gumuz, and Somali Regions. Lowlands of Oromia

and Southern Nations, Nationalities and Peoples, Regions (SNNPR) also grow a significant amount (Terefe *et al.*, 2012)

2.4 Economic Importance of sesame

Sesame is the second largest source of foreign exchange earner after coffee in Ethiopia (Wijnands *et al.*, 2011). It accounts for about 90% of the values of oilseed exports (Kindie, 2007). Accordingly, sesame is increasing the potential of generating income for 171,529 farmers in North Gondar administrative zone who have been cultivating sesame on 129,813.34 Hectare (ha) of land in 2007 main cropping season Central Statistical Agency (CSA, 2015). Sesame plays an important role in human nutrition. Most of the sesame seeds used for oil extraction and the rest are used for edible purposes (Elleuch *et al.*, 2007). It is grown for its seeds, prized oil, and oil paste. The oil paste, tahini, is obtained by grinding the seeds. The seed is also used on breads and cakes. Sesame is useful as an extra rich source of protein in many developing countries (Uzun *et al.*, 2002). Sesame seeds contain 50-60% of the oil. Sesame is known as the queen of oil seeds because its oil not only has nutritive value, but also it contains high quality and quantity (Bedigian, 2004). Lignans antioxidants are present in the oil and are unique for sesame. The lignans sesame and sesamolin and their derivatives prevent oxidation of the oil and give it a long shelf life and stability.

According to research, sesame has many beneficial effects for human health. For instance, scientists showed that sesame leads to the reduction of total serum cholesterol and low-density lipoprotein (LDL) cholesterol and improvement of antioxidant capacity in hypercholesterolemia patients (Chen *et al.*, 2005). Sesame also increases vitamin E concentrations in plasma (Frank, 2005). Shahidi *et al.* (2006) showed significant levels of total phenolic compounds, total antioxidant capacity and free radical scavenging capacity of white and black sesame seeds. Phenolic compounds are very important antioxidants in plants because they can stabilize radical intermediates via donating hydrogen atoms or an electron and prevent the oxidation of various biological molecules.

2.5 Production constraints of sesame

Sesame production in Ethiopia has been increasing extensively in the production area from 64,000 hectares to 420,494 from 2007 to 2015Central Statistical Agency (CSA, 2015).

Despite the country's immense potential to increase Sesame production and productivity and significantly increase the international market's demand for sesame, both the production and marketing system of sesame are full of challenges inhibiting the potential for all involved parties. The level of productivity of sesame (seven quintals/hectare) is by far below 50% of the estimated potential of the country and the average productivity level of other sesame-producing countries (Gelalcha, 2009).

Among the many production constraints, the most important include a lack of improved cultivars, a poor seed supply system and a lack of adequate knowledge of farming and post-harvest crop management and pests (Gelalcha, 2009). Poor management practices, plant population and spacing, planting methods and diseases and insect pests are described as the main production constraints of sesame in Ethiopia. In Ethiopia among the major factors responsible for lower yields, diseases play an important role. Sesame is also affected by many biotic and abiotic stresses. Among the biotic agents, fungi cause major diseases, followed by bacteria, viruses and nematodes. Major Sesame diseases caused by fungi are: Leaf spot/blight (*Alternaria sesami*), *Cercospora* leaf spot (*Cercospora sesami*.), Wilt (*Fusarium oxysporum f.* sp. *sesami*), Root rot (*Rhizoctonia bataticola*), Powdery mildew (*Sphaerotheca fuliginia*), stem blight (*Phytophthora parasitica*) and Anthracnose (*Colletotrichum capsici*); bacterial Leaf blight / spot (*Pseudomonas sesami*); Mycoplasmal such as phyllody (*Mycoplasma*); viral diseases such as Leaf curl (*Nicotina virus)*, Mosaic (Cucumber mosaic virus), Necrosis (Tobacco streak virus) and root knot (*Meloidogyne hapla*) nematode (Gelalcha, 2009; Guleria and Kumar, 2006; Gupta *et al.*, 2018).

2.6 Diseases of Sesame: An overview

2.6.1 *Alternaria* leaf spot

The disease caused by (*Alternaria sesami*) is one of the most common and economically important foliar diseases of sesame. The disease reported in different sesame growing parts of the country by many workers. It affects the plants at all stages and symptoms produce are small dark brown water soaked, round to irregular lesions with concentric rings varying from 1-8 mm in diameter (Gadhi *et al.*, 2018). In severe infections several spots involving major portions of the leaf blade and later drop off from the plants. *Alternaria* blight affects severely at all stages of the crop growth during kharif seasons. The plants were observed to be most

susceptible at 8-10 week's age (Gupta *et al.*, 2018). Dark brown spots are developed on cotyledons, water soaked circular or irregular brown spots on leaves, and brown stripes are formed on stem by the fungus. Resistant varieties are the best option for managing *Alternaria* blight and some resistant sources have also been reported by (Rajput *et al.*, 2013).

2.6.2 *Fusarium* Wilt

Fusarium wilts caused by *Fusarium oxysporum f. spp. sesami* (FOS) is a devastating disease infecting the crop right from seedling to maturity resulting in crop losses in varying degrees depending on the severity of infection. It has been reported as a most important soil borne disease causing severe economic losses on sesame in different countries. The soil borne fungus *Fusarium oxysporum* have a many specialized forms and races that causes *Fusarium* wilts in crop plants. The pathogen *F. oxysporum* can be found in a variety of soil types and also in many any host plants, once the fungus is introduced into a garden, nursery, greenhouse, or field. Thus, a broad range of economically imperative crops is infected by *F. oxysporum* to cause wilt disease (Jyothi *et al.*, 2011). *Fusarium* is an anamorphic species confine by macro and micro morphological descriptors like colony color, conidiophores structure, the presence or absence of micro conidia and Chlamydospores, size and shape of the macro conidia. The most of *Fusarium* species are virulence and cause disease in plants. At least one *Fusarium* -associated disease is found in many crop plants (Leslie *et al*., 2006). The plant diseases such as crown rots, head blights, scabs, vascular wilts, root rots, and cankers were caused by this fungus. The mycotoxin produced by *Fusarium* species affects 25 % of the world food crops and pose a severe threat to plant, animal and human health (Nik, 2008).*Fusarium oxysporum* is asymptomatic fungi and isolated mostly from the roots of crop plants. *Fusarium oxysporum* has an aptitude to stick without choice to pathogenesis. These fungal strains are found in agricultural soils throughout the world and having the nature of pathogenic and non-pathogenic. Mostly, strains of *Fusarium oxysporum* are found in cultivated soils and wild plant systems.

2.6.3 Cercospora leaf spot

The disease is caused by (*Cercospora sesami* Zimm) and is one of the most economically important diseases of sesame in almost all the production area. The crop is affected by the pathogens at all stages of the growth and causes heavy economic losses. Due to lack of resistant sources the released varieties are highly susceptible to *Cercospora leaf spot*. It

appears as small, angular brown leaf spot 5-15 um in diameter on both leaf surfaces. Under favorable conditions, the disease spreads to leaf petiole, stem and capsules producing linear dark coloured lesions. The damage to plant growth and grain yield depend on the severity of infection on the stem and pods and the stage at which the infection takes place. The fungus is seed borne, both internally and externally, but can also survive in the plant debris. Thus, primary infection in the field may be from seed and infested plant debris and secondary spread may be through wind borne conidia. Extensive infection of foliage and capsule leads to defoliation and damage of sesame capsule and yield losses may range from 22 to 53% (Enikuomehin *et al.,* 2006).

2.6.4 *Phytophthora* blight

Phytophthora blight **(***Phytophthora parasitica* var. *sesami***)** produces initial symptoms of water-soaked spots on leaves and stem. The spots are brown in the beginning which later turns to black. Disease can attack at all the stages of the crop (Roy *et al*., 2007).

2.6.5 Bacterial leaf spot

Light brown angular spot with dark purple margin appears in the leaf veins are the symptoms produced by Bacterial leaf spot (*Pseudomonas syringe* pv. *sesame* (Cook 1981). Pseudomonas and other Gram–ve bacterial genera infected plants through natural opening such as tomato and wounds. Multiplying in the intercellular space outside of the plant cell wall and produce virulence factors which contributed to the formation of the symptoms.

2.7 Biology of *Alternaria* leaf spot of sesame

The conidiogenous mycelia or conidiophores of most of the *Alternaria* species produce asexual spores or conidia and measurement ranging between 160 and 200 μm in length. In laboratory conditions, the sporulation occurs at 10–24 °C and maturity of the conidia develops after 14–24 h. The optimum temperature for sporulation ranged between 16 and 24 °C and time up to 12–14 h. The presences of moisture or high relative humidity are very important for sporulation and infection of fungal pathogen propagules. The minimum period of 9–18 hour is crucial for most of the *Alternaria species*. High relative humidity (91.5 %) anda temperature of 20 °C or sometimes in higher range will produce full grown conidiain large quantities in a period of 24 h (Nagrale *et al.*, 2016). The plant pathogenic *Alternaria* species survives as spore and/or mycelium in the diseased crop debris, left over in the fields and in

infected seeds and/or fruits in storage conditions. The etiology of *Alternaria* diseases depends on mode of infection and carrier (fig. 1). In case of seed infection, they cause seedling damage, damping off, stem lesions or cankers, root or collar rot, etc. However, soil moisture and relative humidity play vital role in growth and sporulation of fungi in infected plant residues. Conidia are carried by winds, rain splashes, birds, anthropogenic activities onto healthy plant parts. The fungus can survive in alternate and/or collateral hosts, weeds or perennials in cropping systems. The free water is essential for germination, sporulation and infection of fungi. The active penetration can be occurred on host surface or passively through natural openings. The old and weak host tissues are more susceptible to infection than healthy and vigorous hosts. The *A. japonica* is carried or transmitted by seeds of radish was confirmed by seed germination tests. The pathogen propagules were noticed to present in the seed coat and can be seen in growing seedlings, especially in cotyledons and epicotyl-hypocotyl. Generally, seed coats of most seeds are adhered to hypocotyls axes; hence in some cases the cotyledons may escape infection. The surface sterilization removes 90 % of *A. raphani* from the surface of seeds of radish (Raney *et al.*, 2011; Salehi and Izadpanah, 1992; Sheahan, 2014). The reduction in seed germination was up to 7 % where 35 % seeds may carry the fungus internally, externally or both.

2.7.1 Occurrence, distribution and yield losses of *Alternaria* species

Alternaria leaf spot is prevalent in all the sesame growing areas of the world and it is also reported in Kenya, Ethiopia, El-Salvador, Nigeria, India and USA (Verma *et al.*, 2005). *Alternaria sesami* can cause seed rot, pre and post emergence losses, stem rot and leaf spots. It attacks seedlings, stems of young plants, leaves and pods of *Sesamum indicum* causing considerable damage to plants. The incidence of the disease in the major sesame growing areas ranged from 8 to 92 % (Fula, *2005*) which was influenced by various environmental factors like temperature, light and humidity. It can transmit via seeds and has been reported to be of worldwide distribution. Its occurrence in epidemic proportions has been reported from the United States (Culp and Thomas, 1964), Ethiopia (Ellis and Holliday, 1970), Salvador(Weiss, 1971), India (Deshpande and Shinde, 1976) and Kenya (Gatumbi, 1986).The pathogen can survive between cropping seasons or unfavorable conditions as an infection in the seeds (Kolte, 2018). Mohanty and Behera (1958) reported first in India the occurrence of *Alternaria* leaf blight on sesame (*Alternaria sesami*). On leaves, initially small brown spots appear which

later enlarge, darker in color with concentric zonation. On undersurface of the leaves, the spots are grayish brown in color. In severe infections, spots coalesce covering a large portion of the leaf, which ultimately dry and shed off. Severely affected plants fail to produce flowers.

2.7.2 Disease cycle of *Alternaria* species

The disease symptoms were mainly found on the leaf blade as brown, round to irregular spots varying from 1 to 8 mm in diameter (Fig.1). In the early stages of infection, minute brown spots appear on the leaf blades which later become darker in color with concentric zonation demarcated with brown lines inside the spots on the upper surface. Mohanty and Behera (1958) reported first in India the occurrence of *Alternaria* leaf blight on sesame (*Alternaria sesami*). The spots appear on cotyledons, leaves, stems and capsules. On leaves, initially small brown spots appear which later enlarge, darker in color with concentric zonation. On undersurface of the leaves, the spots are grayish brown in color. In severe infections, spots coalesce covering a large portion of the leaf, which ultimately dry and shed off. Severely affected plants fail to produce flowers.

Berry (1960) reported that *Alternaria sesami* cause seedling damping-off and also causes considerable damage to sesame capsules. Dolle (1981) reported that visible symptoms of *Alternaria* leaf spot disease of sesame seedlings appeared at 3 days after germination and disease development reached its peak when the crop was 35 days old. Kolte (1985) described the symptoms of sesame *Alternaria* leaf blight (*Alternaria sesami*) as initially small, irregular, brown spots on leaf blades, which later enlarged and coalesced forming elongated lesions. Similarly, symptoms also appeared to stem and capsule. Severe infection caused complete defoliation Naik *et al.* (2004) reported *Alternaria* leaf blight (*Alternaria sesami*) as an economically important disease of sesame causing seed rot, pre and post emergence death of seedlings, resulting in considerable qualitative and quantitative losses.

Gulariya and Kumar (2006) reported the symptoms induced by *Alternaria sesami* in sesame as small, circular reddish-brown spots (1-8 mm) on leaves which enlarge later with concentric rings covering the entire leaf area. Khati and Pandey (2008) noticed the symptoms of sesame leaf blight/spot (*Alternaria sesami*) in Kumauni hills as initial minute, round to irregular dark brown zonate spots (8 - 10 mm diameter), which later enlarged rapidly, coalesced and caused lightening of leaves which become papery and fall prematurely.

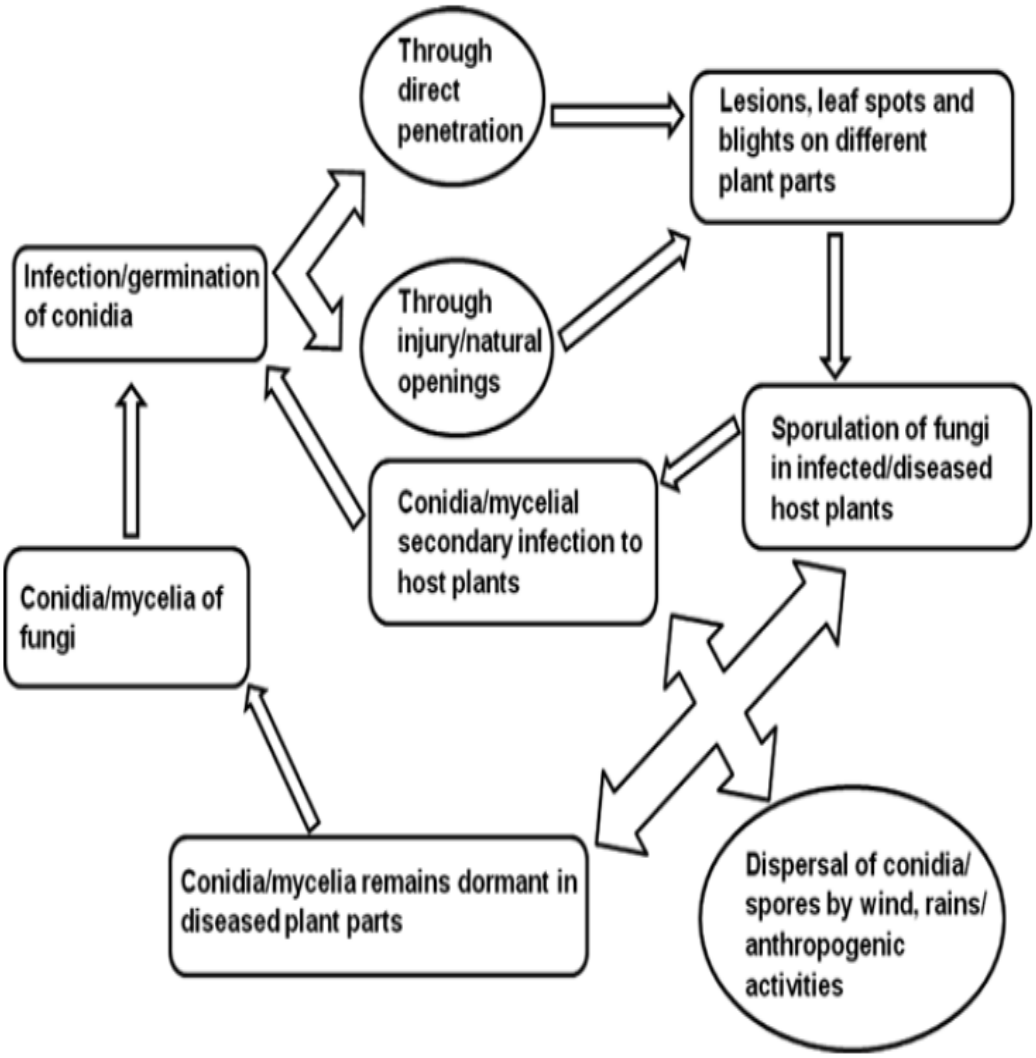


Fig. 1: Disease cycle of phytopathogenic *Alternaria* species (Raney *et al.*, 2011)

2.7.3 Cultural and morphological variability

It is very important to know the existence of variability among population of pathogens. Also, information on pathogenic, cultural, morphological and molecular variability of the pathogens helps in the selection of more virulent strains for identification of host resistance. Therefore, pathogenic, cultural, morphological and molecular variability among the isolates/populations of *Alternaria sesami* (sesame) and other *Alternaria* species especially, infecting oilseed crops, which reported earlier by several workers are being summarized in the following paragraphs (Kindie, 2007).

Simmons (2004) reported that colonies of *Alternaria* sesami on V-8, PCA and Hay agars. Sporulation was dense on V-8, abundant on PCA and Hay. Concentric rings of growth and sporulation are evident on V-8. Conidiophores, simple or sparingly branched; which measured 60-225 x 5.0- 6.5 µm. Juvenile conidia were narrow-ellipsoid, tapering to a pointed apex. Young conidia were ellipsoid or ovoid, bearing the beak. Transverse and longitudinal septum formation was abundant and obvious. Large conidium size ranged from 100-130 x 19-32 µm, with 10-12 transverse septa and 1-3 long septa. Conidium color diluted dull brown with darker major septa.

Naik *et al.* (2004) studied cultural and morphological variability among 164 isolates of *Alternaria* spp (*A. alternata, A. sesami*) causing leaf blight of sesame crop. Colony color of varied form of gray to light brown and both fluffy mycelium (37) and smooth type (30) of with regular to irregular colony margin. The majority of the isolates were fast growing (119) and some with moderate (45) growth. Twenty five percent of the isolates (40 isolates) were highly sporulating and some of them (54) were shy in sporulation. Individual isolates were studied in detail on the type of growth margin, color of the colony, radial growth, sporulation, the width of the mycelium, vertical (0-3) and horizontal (2-5) septations of conidia, size of conidia (33.1-196 × 24.4-78.6 µm) and length of beak (7.1-88 µm).

2.8 Biology of *Fusarium* wilts of sesame

Fusarium wilts caused by *Fusarium oxysporum f. spp. Sesami* (FOS) is a devastating disease infecting the crop right from seedling to maturity resulting in crop losses in varying degrees depending on the severity of infection. It has been reported as a most important soil borne disease causing severe economic losses on sesame in different countries (Kumar, 1992). As it is a soil borne disease and once noticed in the field cannot be easily controlled by any means, insulation of agronomically superior varieties with genetic resistance to the disease is therefore, the best means to manage it and thereby minimize the yield losses. Unfortunately, very little is known of the existence of reliable sources of resistance (Chung and Hong, 1991). *Fusarium oxysporum of Sesami* (FOS) is one of the most important soil borne fungal diseases infecting on root, stem and foliar components and causes economic yield loss in different countries.

Wilt disease of sesame (*Sesamum indicum* L.) caused by *Fusarium oxysporum* f. spp. *Sesami* is the most serious diseases causing losses in seed yield in Egypt (Ziedan *et al.*, 2011). The soil-borne fungus *Fusarium oxysporum* is the causal agent of vascular wilt disease resulting in sudden death of sesame plants. This pathogen may cause heavy yield losses ranging from 50–100%.*Fusarium oxysporum* is an important vascular wilt pathogen on many plant species and is also responsible for many damping-off diseases, and crown and root rot. The fungus can be found worldwide and is considered as the most widely dispersed and most economically important *Fusarium* species (Leslie and Summerell, 2006).

2.8.1 Distribution and Diversity of *Fusarium* Wilt of sesame

Presently, about 80% of plant diseases can be traced to fungal pathogens. *Fusarium* wilt is a soil-borne fungal disease in which the water-conducting (xylem) vessels become blocked, so that the plant wilts and often dies. *Fusarium* wilts are caused by pathogenic strains of several species of *Fusarium*, including *F. eumartii, F. oxysporum, F. avenaceum, F. solani, F. sulphureum and F. Tabacinum* are usually very host-specific. However, the most commonly encountered culprit is *F. oxysporum*. Vascular wilts are widespread in distribution causing tremendous losses in most kinds of vegetables, flowers, field crops, perennial, ornamentals, and fruit and forest tree (Agrios, 2005). Economically wilt is the most important disease of broad bean. Losses could be tremendous, especially in seasons when hot spells prevail just after germination. Affected plants are either killed or, if survived, show greatly reduced growth. The pathogen (*Fusarium oxysporum*) causing wilt is soil borne and is present all over the growing area, but only attack whenever suitable conditions (mainly high temperature) prevail (Zhang *et al.*, 2012).

2.8.2 Disease cycle of *Fusarium* wilts

Fusarium oxysporum is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al*., 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types. Healthy plants can become infected by *Fusarium oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant, either with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the

root tips, through wounds in the roots, or at the formation point of lateral roots. Once inside the plant, the mycelium grows through the root cortex intracellular. When the mycelium reaches the xylem, it invades the vessels through the xylem's pits. At this point, the mycelium remains in the vessels, where it usually advances upwards toward the stem and crown of the plant (Agrios, 2005). Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly. The resulting spores can then be used as new inoculum for further spread of the fungus.

2.8.3 Morphological and cultural characters of Fusarium species

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple - according to the strain (or special form) of *Fusarium oxysporum.* If sporodochia are abundant, the culture may appear cream or orange in color (Smith *et al*., 1988). *Fusarium oxysporum* produces three types of asexual spores: microconidia, macroconidia, and Chlamydospores (Agrios, 2005). Microconidia are one or two celled, and are the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. These spores are either one or two celled (Agrios, 2005).

2.9 Management of sesame diseases

2.9.1 Cultural practices

Soil amendment with farm yard manures is helpful in reducing the incidence of the disease. Avoidance of planting overlapping crops in adjacent, crop rotations Viz. Sesame Maize-Cabbage, Okra-Sesame-Maize, Maize-Sesame-Maize are reported to be effective in reducing disease incidence. Crop rotation with non-host crops, particularly with rice, not only help

reducing the disease incidents but also in proving good drainage (Anonymous, 1998). Destruction of crop debris, removal of alternate weed host, providing irrigation at critical stages of the crop, an avoiding water logging and water stress during lowering stage help controlling pest and diseases. Avoidance of chemical spray when 1-2 larval parasitoids are observed help to enhance parasitic activity.

2.9.2 Chemical methods

For the prevention of seed borne diseases, use the seed essentially treated with Thiram (1.5 g) + Bavistin (1.5g). Wherever bacterial leaf spot disease is a problem, soak the seed for 30 minutes in 0.025% solution of Agrimycin prior to seeding. El- Khadem *et al.,* 1991 found that Benlate fungicide was found most effective. Khlifa, 1997 reported also that Rizolex-f or Benlate as seed treatment +Chlorneb as soil treatment were superior for controlling root rot and wilt diseases and increased seed yield of sesame under field conditions.

2.9.3 Uses of resistant varieties

Development of a resistant variety requires the knowledge of genetics and inheritance of the disease resistance. However, there are limited studies reporting the inheritance of phyllody resistance in sesame. Sing *et al*. (2007) reported that a single recessive gene governs resistance in cultivated varieties whereas, wild species possess a single dominant gene conferring resistance against phytoplasma. In most of the cultivated genotype were found to be highly susceptible to *Alternaria* leaf blight expect RT-273 and some promising crosses were advanced to F7 (Naik *et al*., 2003) and wild species like *S. radiatum, S. prostatum, S. lacinietum, S. mulayanum and S. occidentale*var. malabaricum were found to be resistant. Breeding for disease resistance through conventional approach is still in infancy (Kariyallappa *et al*., 2003), mainly because of very few resistant genotypes identified in *Sesamum indicum* types. Field screening of germplasm, either indigenous or exotic, by several workers has resulted in availability of very few resistance genotypes (Naik *et al.,* 2003). As varietal replacement is able to provide a spatial and temporal discontinuity against the pathogen, therefore, use of resistant varieties has been the main approach in disease control. The use of resistant varieties is the cheapest, easiest, safest and most effective means of controlling plant diseases in crops for which some varieties are available.

2.9.4 Systemic acquired resistance

The plant disease management through induced systemic resistance by the application of biotic and abiotic agents is one of the important strategies under ecofriendly disease management. Under abiotic activators of inducing host resistance against plant pathogens, application salicylic acid, jasmonic acid and phosphatic salts are common. Klessig and Malamy (1994) reported that chemicals like salicylic acid, jasmonic acid, cow urine and microbial bioagents like *Pseudomonas* sp. induces systemic host resistance with challenge inoculation of these agents/ compounds in certain quantities. Ratnam *etal*. (2000) observed that sunflower seeds when treated with salicylic acid (5 mM) and bion (5 mM) induces systemic host plant resistance as evidenced from higher phenol content in host plant and recorded reduced disease severity.

2.10 Biological Methods

2.10.1 Botanicals

The use of plant extracts for controlling *Fusarium* species, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Chand and Singh(2005) reported that the plant extracts, 13 VIZ Calotropis procera, Eucalyyptus globulens, Jatropha multifida, Azadirachta indicia, Allium sativum were significantly pronounced in reducing wilt incidence in Cicer arietinum L. Mycelial growth of various Fusarium species were inhibited by the plant extracts of Adhatodavasica, Azadirachta indica ,Cinnamomum camphora,and Ocimum sanctum (Prasad and Ojha,1986);Agave Americana, Cassia nadosa Redd and Reddy, (1987); Azadirachta indicia (Eswaramoothy *et al*., 1989); Azadirachta indica, Atrophabella donna, Calotropisprocera, Eucalyptus amgdalline, Ailanthus exclsa and Lantana camera (Bansal and Rajesh, 2000). Also Singh, (2004) reported that Leaf etract of Azadirachtaindica at 100/con completely inhibited germination of pathogen spores.

2.10.2 Antagonistic Microbes

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 pest or pathogen is referred to as the biological control agent (BCA) (Benítez *et al.*, 2004). 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 the mass introduction of one or more antagonists. 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 (Bunbury-Blanchette and Walker, 2019; Degenkolb *et al.*, 2015). With regards to plant diseases, suppression can be accomplished in many ways. Biological control refers to the purposeful utilization of introducing or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens. This may involve the use of microbial inoculants to suppress a single type or class of plant diseases. Or, this may involve managing soils to promote the combined activities of native soil and plant associated organisms that contribute to general suppression.

2.10.2.1 *Trichoderma* species

Trichoderma has long been recognized as agents for the biocontrol of plant diseases. The potential of *Trichoderma* species as biocontrol agents of plant pathogens was first recognized in the early 1930s (Weindling, 1932). *Trichoderma* species can directly affect mycelia or survival propagules of other fungi through the production of toxic secondary metabolites, formation of specialized structures, and secretion of cell wall-degrading enzymes (Sarrocco *et al.*, 2006). *Trichoderma* species are very useful filamentous fungi. By producing beneficial effects on crops, they have actually sustained the agricultural yields that have supported the human population over the millennia. Together with other beneficial microbes, they help to maintain the general disease suppressiveness and fertility of soils, and aid in the maturation of compost for natural fertilizer production (Harman *et al*., 2004).

Trichoderma species are ubiquitous and often predominant components of the mycoflora in native and agricultural soils throughout all climatic zones. They colonize aboveground and belowground plant organs and grow intercellular (endophytes), and they appear in plant litter, soil organic matter (saprophytes), and mammalian tissues (human pathogens). However, the

ability of these fungi to recognition, invade, and destroy other fungi has been the major driving force behind their commercial success as bio pesticides. These fungi not only protect plants by killing other fungi and certain nematodes, but induce resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, solubilize plant nutrients, and bio-remediate heavy metals and environmental pollutants. Better understanding of how *Trichoderma* evolved to interact with other fungi and wild plants will improve and expand their applications. The ability to attack other fungi, most importantly soil borne plant dominated the interest in *Trichoderma* for many years. Recent years have witnessed a wave of interest in plant disease resistance, namely induced systemic resistance (ISR); systemic acquired resistance (SAR) induced by the *Trichoderma* -root symbiosis. These plant-centered mechanisms have rivaled *mycoparasitism* as an explanation for how *Trichoderma* controls plant diseases. The genome sequencing of *Trichoderma* species has stimulated the development of systems, biological approaches, initiated and enhanced whole-genome expression studies, and provided unique data for phylogenetic and Bioinformatics analyses toward understanding the roles of these opportunists in ecosystems (Mukherjee *et al.*, 2012; Mukherjee *et al.*, 2013).

At present the, genome sequences of seven species: *Trichoderma reesei, T. Viernes, T. atroviride, T. harzianum, T. asperellum, T. longibrachiatum,* and *T. citrinoviride* are available (Grigoriev *et al*., 2012). The genome of *Trichoderma* species has been extensively investigated and has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt too many different environments (soil, water, dead tissues, inside the plant). Several laboratories have recently started or planned to use proteomic and functional genomics analysis in the attempt to obtain an overall picture of the changes that occur in the *Trichoderma,* plant, and pathogen express some when they “talk” to each other, especially when an increase in disease resistance is generated. *Trichoderma* species are the most successful bio-fungicides used in today’s agriculture with more than 60 % of the registered bio fungicides world-wide being *Trichoderma* - based (Verma *et al*., 2007). In India alone, about 250 products are available for field applications (Singh *et al*., 2009). Despite this remarkable success, the share of bio-fungicides are only a fraction of the fungicide market, dominated by synthetic chemicals. The

major limitations of microbe-based fungicides are they restricted efficacy and their inconsistency under field conditions. The origin of these difficulties is that microbes are slow to act, compared to chemicals, and is influenced by environmental factors.

2.10.2. Morphology and distribution of Trichoderma species

Trichoderma is filamentous fungi commonly found in the soil community that are facultative saprophytes. They are members of a genus belonging to a group of largely asexually reproducing fungi that include a wide spectrum of micromycetes that range from very effective soil colonizers with high biodegradation potential to facultative plant symbionts that colonize the rhizosphere (Khalid, 2009). *Trichoderma* is usually recognized by the presence of fast-growing colonies producing white, green, or yellow cushions of sporulating filaments, the fertile filaments or conidiophores produce side branches bearing whorls of short phialides that support the spherical to ovoid green colored *Trichoderma* is found in nearly all temperate and tropical soils, where samples contained 10^1-10^3 cultivable propagules per gram of soil. These fungi also colonize woody and herbaceous plant materials, in which the sexual teleomorph (Genus *Hypocrea*) most frequently found *(*Khalid, 2009*)*

Trichoderma has rapid growth and development, and also produces a large number of enzymes, induced by the presence of phytopathogenic fungi. Its high tolerance to extreme environmental conditions and habitat, where fungi are the cause of various diseases, makes it an efficient agent of control; equally, it can survive in media with high levels of pesticides and other chemicals. So the application of *Trichoderma* species directly on the soil offers greater protection to the crops (Cooney and Lauren, 1998). The mechanisms that *Trichoderma* species uses to antagonize phytopathogenic fungi include competition, colonization, antibiosis and direct mycoparasitism (Howell, 2003). This antagonistic potential serves as the basis for effective biological control applications of different *Trichoderma* strains as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Chet, 1987).

Trichoderma species stimulates plant growth by producing substances that stimulate plant growth and development. These substances act as catalysts or accelerators in the primary meristem tissues in the younger parts of plants, accelerating cell reproduction, so that the plants achieve faster growth than those which have not been treated with this microorganism (Dennis and Webster, 1971).

2.10.3. Trichoderma -Plant Interactions

Many *Trichoderma* species grow in the rhizosphere and are capable of penetrating and internally colonizing plant roots (Harman *et al.*, 2004). This opportunistic/facultative symbiosis is driven by the ability of *Trichoderma* to derive sucrose or other nutrients from plants, in return for boosting plant immunity against invading pathogens. The presence of *Trichoderma* in the rhizosphere evokes a coordinated transcriptomic, proteomic and metabolic response in the plants (Shoresh and Harman, 2008). This reprogramming of the plant is often beneficial, improving growth, yield and resistance to pathogens. The combined ability to attack soil-borne pathogens while priming plant defenses, however, is what promotes *Trichoderma* as such a promising partner for sustainable management of plant diseases.

2.11. Mechanism of Action of *Trichoderma* Species

Of the bio-control agents, *Trichoderma* species has demonstrated effective and selective enough against most of the fungal diseases. *Trichoderma* species have developed numerous mechanisms by which they are attacking other fungi. These mechanisms include mycoparasitism (Haran *et al*., 2004), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), competition for space and nutrient, inactivation of the pathogen's enzymes and induced resistance. Today, more than 50 different *Trichoderma* -formulated agricultural products can be found and are sold and applied to protect and improve the yield of fruits, vegetables and ornamentals (Lorito, 2005). *Trichoderma* is completely safe to the humans, animals and the environment.

2.11.1 Attachment to Host

Attachment and infection of host fungi by mycoparasitism *Trichoderma* is accompanied by the formation of appressoria and/or mycoparasitism (Druzhinina *et al.*, 2011). The genetic studies underlying attachment of the pathogen to the host are not well understood, although proteins like structures are possibly involved. Though experimental evidence is lacking, indirect support for the involvement of hydrophobins comes from the finding that *T. viren s*mutants in the transcriptional regulator of secondary metabolism and morphogenesis, which have decreased hydrophobins expression, were defective in both hydrophobicity and mycoparasitism (Mukherjee and Kenerley, 2010).

2.11.2 Killing the Host

Trichoderma species produces lytic enzymes (chitinase and glucanase) and antibiotics, thereby kill other fungi. Not surprisingly, the genomes of the mycoparasitic *Trichoderma* species are rich in genes encoding enzymes like chitinase and glucanase, and those for secondary metabolism like NRPSs (Kubicek *et al.*, 2011). Glucanase is another group of cell wall-lytic enzymes with roles in mycoparasitism/ biocontrol. Deletion of tvbgn3 (β-1, 6-glucanase-encoding) reduced the mycoparasitic and bio control potential of *T. virens* against *P. Ultimum* (Viterbo and Horwotz, 2010). In addition to chitinase and glucanase, proteases like Prb1/Sp1 are induced during mycoparasitism and play definitive roles in biocontrol (Djonovic*et al.*, 2006).

2.11.3 Antibiosis

Antibiosis occurs during interactions involving low-molecular-weight diffusible compounds or antibiotics produced by *Trichoderma* strains that inhibit the growth of other microorganisms. Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that impede colonization by antagonized microorganisms; among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptides, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described (Vey *et al*., 2001). In some cases, antibiotic production correlates with biocontrol ability, and purified antibiotics mimic the effect of the whole agent. However, there are also examples of antibiotic-overproducing strains, such as gliovirin overproducing mutants of *T. virens*, which provide control similar to that of the wild-type, and of gliovirin- deficient mutants which failed to protect cotton seedlings from *P. ultimum*, whereas the parental strain did (Chet *et al*., 1997). In general, strains of *T. virens* with the best efficiency as biocontrol agents are able to produce gliovirin (Howell 1998). Also, the most effective isolates of *T. harzianum* against *Gaeumannomycesgraminis var. tritici* produce pyrone antibiotics, and the success of the strains was clearly related to the pyrone they produced. Peptaibols-a class of linear peptides that generally has strong antimicrobial activity against gram-positive bacteria and fungi-act synergistically with cell-wall-degrading enzymes (CWDEs) to inhibit the growth of fungal pathogens and elicit plant resistance to pathogens (Wiest *et al*., 2002). In tobacco plants, exogenous applications of peptaibols trigger a defense response and reduce susceptibility to tobacco mosaic virus (Wiest *et al*., 2002). A peptaibols synthetase from *T. virens* has recently

been purified, and the corresponding gene, which has been cloned, will facilitate studies of this compound and its contribution to bio control. An extensive review on antibiosis and production of *Trichoderma* secondary metabolites is provided in Howell (Howell, 2003).

2.11.4 Competition

Trichoderma species are generally considered to be aggressive competitors, grow very fast and rapidly colonize substrates to exclude pathogens such as *Fusarium* species (Papavizas 1985). Rhizosphere competence, following seed treatment is an important strategy to create a zone of protection against plant pathogens (Howell, 2003). *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plants (Ahmad and Baker, 1987). Soil application with *T. harzianum* spores inhibited the infestations of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis.* Competition was a proposed mechanism, although it was not proven to be the main activity.

2.11.5 Mycoparasitism

In contrast to studies on hyphal parasitism, very little research has been done on the molecular mechanisms of parasitism of resting structures. *Trichoderm*a species are prolific producers of secondary metabolites and the genomes of the mycoparasitic *Trichoderma* species are especially enriched in genes for secondary metabolism (Reino *et al*., 2008). Roles of antimicrobial secondary metabolites such as gliotoxin and gliovirin in suppression of *R. solani* and *P. ultimum*have been suggested, although contradictory reports exist (Viterbo and Horwotz 2010). Certain species like *T. atroviride* produce the volatile metabolite which plays an important role in *Trichoderma* –plant and *Trichoderma* -fungal interactions.

2.12 Formulation and application methods of biological control agents

Production, formulation and application of biocontrol agents (BCA) Production, formulation and application of BCAs have been investigated extensively with the aim of producing successful and cost-effective products (Hall and Menn, 1999). A major aim is to produce the greatest quantity of viable propagules with the best quality for formulation as cheaply as possible, preferably using inexpensive growing media such as industrial wastes. Production of bacteria and fungi can be done using large-scale liquid fermentation which often involves manipulating the culture medium to induce production of the desired propagules for formulation. Factors which are often manipulated include temperature, pH and osmotic

potential, as well as nutritional factors such as carbon source and C: N ratio (Jackson, 1997). Recently, solid-state fermentation has been used for the production of fungal biomass. For example, conidia produced by solid-state fermentation are incorporated into the wettable granule formulation of the commercial *C. minitans* product, Contans WG (De Vrije *et al*., 2001).

2.13 Methods of application of antagonists

2.13.1 Overall application

Successful application of biological control strategies requires more knowledge-intensive management (Heydari *et al*., 2004). Understanding when and where biological control of plant pathogens can be profitable, requires an appreciation of its place within integrated pest management systems (Shah-Smith and Burns, 1997). In general, the foundation of a sound pest and disease management program in an annual cropping system begins with cultural practices that alter the farm landscape to promote crop health (Heydari *et al*., 2004). These include crop rotations that limit the availability of host material used by plant pathogens (Cook, 1993). Proper use of tillage can disrupt pathogen life cycles and prepare seed beds of optimal moisture and bulk density. Careful management of soil fertility and moisture can also limit plant diseases by minimizing plant stress (Cook, 1993). In nurseries and greenhouses environmental control can be more tightly regulated in terms of temperature, light, moisture and soil composition, but the design of such systems cannot wholly eliminate disease problems (Paulitz and Belanger, 2001). The second layer of defense against pests consists of the quality of crop germplasm. Breeding for pathogen resistance including fungal pathogens contributes substantially to crop success in most regions (Cook, 1993). Newer technologies that directly incorporate genes into crop genomes, commonly referred to as genetic modification or genetic engineering, are bringing new traits into crop. Other technologies, such as seed washing, testing for pathogens and treatments are also used to keep germplasm pathogen-free. In perennial cropping systems, such as orchards and forests, germplasm quality may be more important than cultural practices, because rotation and tillage cannot be used as regularly (Cook, 1993). Upon these two layers, growers can further reduce pathogen pressure by considering both biological and chemical inputs. Biologically based inputs such as microbial fungicides can be used to interfere with pathogen activities. Registered biofungicides are generally labeled with short reentry intervals and pre-harvest intervals,

giving greater flexibility to growers who need to balance their operational requirements and disease management goals. When living microorganisms are introduced, they may also augment natural beneficial populations to further reduce the damage caused by targeted pathogens (Heydari *et al*., 2004).

2.13.2 Applying to the infection site

Application directly to the infection court at a high population level to swamp the pathogen (inundate application), seed coating and treatment with antagonistic fungi and bacteria, e.g., *Trichoderma* harzianum and *Pseudomonas fluorescens* (Heydari *et al*., 2004), antagonists applied to fruit for protection in storage, e.g., Pseudomonas fluorescens (Janisiewicz and Peterson, 2004) and application to soil at the site of seed placement (Heydari, 2003). These types of applications are the most commonly used procedures which have resulted in the successful control of several fungal plant pathogens.

2.13.3 One place application

In this procedure, biocontrol microorganisms are applied at one place (each crop year), but at lower populations which then multiply and spread to other plant parts and give protection (augmentative application) against fungal pathogens. An Example of this method is Plant Growth Promoting Rhizobacteria (PGPR) and a toxigenic Aspergillus flavus on wheat seed scattered on the soil to spread to cotton flowers where they displace Aflatoxin producing strains of A. flavus and fungal antagonists added to soil (Islam *et al*., 2005).

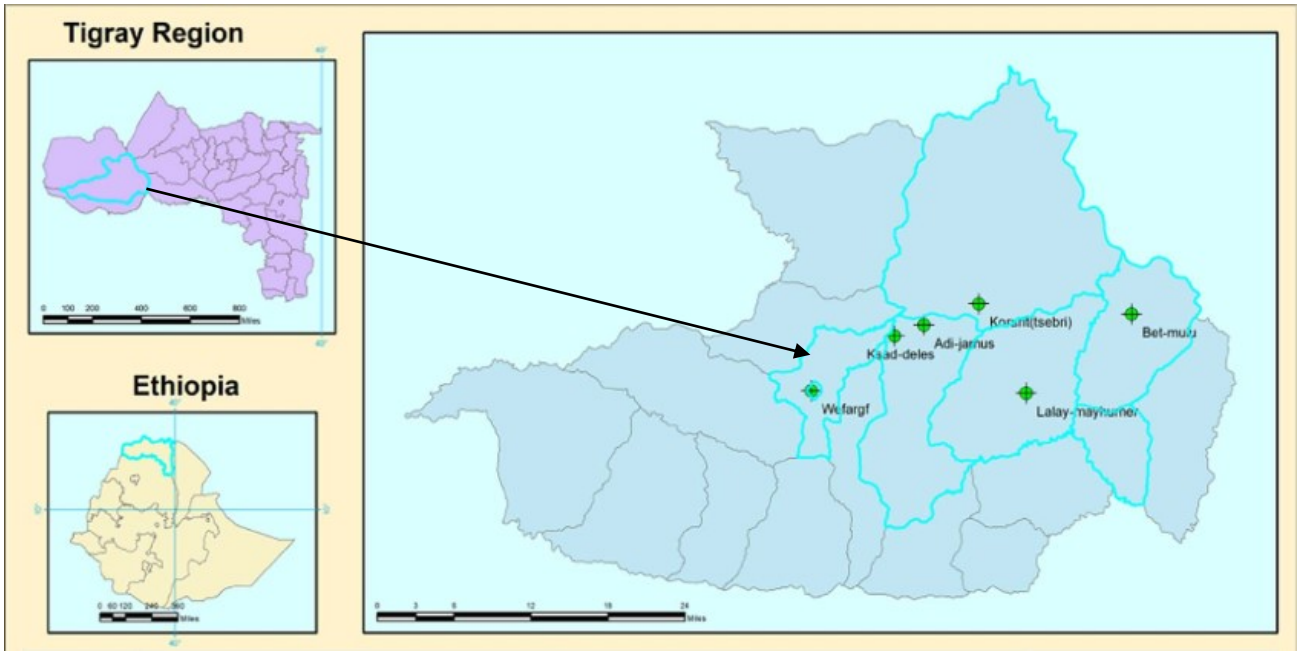
2.13.4 Occasional application

One time or occasional application maintains pathogen populations below threshold levels. In theory, parasites of the pathogen, or hypo virulent (disease carrying) strains of the pathogen, might be used and not require yearly repetition (e.g., hypo virulent strains of the chestnut blight pathogen) in which host plant is inoculated with attenuated strains of pathogenic that protects the host plant against the virulent strains of pathogen (Milgroom and Cortesi, 2004).

3. MATERIALS AND METHODS

3.1 Description of the study areas

The study was conducted in Wolkait district, western zone, Tigray region, Ethiopia. Wolkait is located at 437 km West and 1220 km northwest from the capital city of Tigray region (Mekelle) and country capital city (Addis Ababa), respectively. It has coordinates of 13°30′00″ and 14°07′00″ North latitude, and 36°40′15″ and 37°48′00″ East longitude with an altitude that ranges from 677 to 2755 m above sea level. The district has 28 kebeles of which 14 are with lowland agro-ecology (Fig. 2). The annual temperature and unimodal rainfall of the district are 17.5–25 °C and 700–1800 mm, respectively (Office of Plan and Finance Wolkait district, 2015). Wolkait district is known for its fertile alluvial soil, which grows cash crops such as sesame, cotton and sorghum.



Legend: Study sites/kebeles

Fig. 2. Map of the study area, Wolkait District, Western Tigray, Ethiopia

3.2 Sample collection

Sesame leaves and seed showing visible symptoms of fungal pathogens and soil of each sesame sample were collected purposively from three different Kebeles of Wolkait woreda namely Bet-Mulu, Kalema and Lalay Mayhumer. For each sampling site, sample collections were made from three fields' sites/kebeles within 15-20 km distance car stop. The numbers of samples collected were 15 leaf samples, 15g soil samples and 15 seed samples, five samples from each Kebele. The collection sites represented different geographic locations at varying elevations and with different climatic conditions.

Visual observations were made for the manifestation of the typical symptoms on field growing sesame plants and the leaf samples were carried in khaki paper bags, endorsed with relevant information and brought to the Mycology laboratory, at Addis Ababa University for further studies. The top layer of a soil litter and the upper soil horizon (4–6 cm) was discarded, and 100g of soil from approximately 10cm depth was collected, placed in polyethylene bags and labeled. Afterwards, similar samples from one subarea were merged in several larger samples of the same place, which were, subsequently, sieved and dried on sterile paper for 2-3 days. Moreover, sesame seeds with defects were also collected in kaki paper from each study site. The isolation and identification of fungal pathogens were conducted at Addis Ababa University, College of Natural and Computational Sciences, Department of Microbial, Cellular and Molecular Biology, Addis Ababa, Ethiopia.

3.3 Isolation of Sesame fungal pathogens

Ten grams of soil samples (pulverized by means of a mortar and pestle, and passed through a 0.5 mm soil screen mesh to remove large debris and root fragments) were suspended in 90 ml sterile distilled water and thoroughly mixed. A 1 ml aliquot suspension of soil was taken from a dilution of 10^{-1} used to suspend in 9 ml of sterilize distilled water to prepare a series of dilutions in the range of 10^{-2} to 10^{-3}, and 1ml final suspension was inoculated on Czapek Dox's Agar medium supplemented with streptomycin (250 mg/L to prevent bacterial growth). Each soil sample was replicated three times. Plates were incubated, at 25^{o}C for a period of 7 days and examined daily for colony growth and development. Sesame pathogens were subsequently transferred to grow on PDA medium (Aneja, 2005).

Samples of infected sesame leaf specimens showing typical symptoms of fungal pathogen and seeds were washed thoroughly with distilled water; blot dried. The leaves were cut with a sharp sterilized blade into small bits (1cm^2), keeping half healthy and half diseased portion intact. Then, leaves and seeds were surface sterilized with 2% (percent) of sodium hypochlorite (NaClO) solution for 30 second, then rinsed in three sequential with sterile distilled water in Petri plates to remove traces of sodium hypochlorite and again blot dried. Later, leaf bits and seeds were inoculated aseptically on autoclaved and cooled potato dextrose agar (PDA) medium in sterilized Petri plates under Laminar-air-flow cabinet and incubated at 25^{o}C. Through frequent sub-culturing, the test pathogens were purified and the pure cultures were maintained on a PDA slant in glass test tubes, at the 4^{o}C for further study.

3.4 Identification of sesame fungal pathogens

Sesame fungal pathogens were identified using their growth and cultural characteristics, morphological and microscopically features as described by Kunwar and Manoharachary (2006). The sesame fungal pathogens were characterized based on the following parameters such as pigment production, colony color, spore or conidia producing structures and spore shapes. The spores/conidial, asexual fruiting structures characteristics were studied under compound microscope of 400x magnification (Olympus Microscope, Germany). These characteristics were used in identifying the fungal pathogen isolates to the genus level following standards described by Mathur and Kongsdal (2003).

3.5 Slide culture Technique

A clean slide was placed in 9 cm diameter plates and then a small amount of autoclaved melted PDA medium was put over the slide to make a thin PDA film. Five mm disc of the isolated pathogen isolates were placed on slide. Distilled water was poured in Petri plates to avoid drying (incubated, at 28 +2^{o}C for 5 days. The growth was observed microscopically (Olompus Microscope, Germany) by staining with lactophenol cotton blue (Schuster and Schmoll, 2010).

3.6 Sources and designation of *Trichoderma* isolates

Trichoderma isolates were obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University (AAU). All the *Trichoderma* isolates used in this study were previously

isolated from soil collected from coffee, cotton and faba bean growing areas of farms which represent different agronomical management practices and levels of soil fertility (Table 1). The seven potential *Trichoderma* isolates designated as Addis Ababa University *Trichoderma* -11 (AUT-11), AUT-12, AUT-32, AUT-33, AUT-97, AUT-131 and AUT-158 were used in this study. All fungal isolates were preserved, at 4°C on potato dextrose agar (PDA) slants for further study.

Table 1. *Trichoderma* isolates studied, location and substratum

S/No	*Trichoderma* Isolates	Substratum	Location	Collection year
1	AUT-11	Faba bean farm, soil	Sheno	2019
2	AUT-12	Faba bean farm, soil	Fiche	2019
3	AUT-32	Coffee rhizosphere	Gera	2017
4	AUT-33	Faba bean, soil	Ambo	2019
5	AUT-97	Coffee rhizosphere	Jimma	2018
6	AUT-131	Cotton farm, soil	Wolkait	2019
7	AUT-158	Coffee rhizosphere	Teppi	2018

3.7 Physiological Characterization of *Trichoderma* Isolates

3.7.1 Growth at different Temperatures

The ability of *Trichoderma* isolates was grown on PDA medium at 4, 25 and 37^{o}C and also incubated for 10 days (Hermosa *et al.*, 2012).

3.7.2 Biomass production and spore yield determination of *Trichoderma* species at different pH values

The ability of *Trichoderma* isolates to grow at pH 4.5, 6.5 and 7.5 were tested in potato dextrose broth (PDB) medium. For biomass production Erlenmeyer flask (250ml) containing 50 ml of each PDB medium was inoculated with four mycelial plugs disc (5mm in diameter) of the *Trichoderma* isolates taken for seven days old cultures on PDA. The flasks were plugged with cotton under aseptic conditions and incubated under an incubator at 25^{o}C for 10

days. The treatment was replicated three times. The culture was harvested finally from each replicate. The fungal biomass yield was assessed by collecting fungal biomass on pre-weighed filter paper. The dry weight was determined after 48 hours of oven drying at 65^{o}C. Number of conidia per mg of the biomass was determined by the dilution method with the aid of Haemocytometer.

3.8 . Pathogenicity test

The pathogenicity test was conducted with the cultures that were grown on PDA for seven days. Pathogenicity of two fungal pathogens isolates *(Fusarium* sp and *Alternaria* sp*)* of sesame *was* tested on detached sesame leaves. The suspension of conidia of each pathogen was prepared by suspending mycelia scraped from 7 days old culture, grown in 10 ml of sterile distilled water and stirred vigorously for 90 seconds and then filtered through two-layer cheese cloth. The concentration of spore suspensions was adjusted to 1×10^{5} spore per ml by using Haemocytometer before inoculation. Apparently healthy leaves of sesame grown under green house were collected, washed and sterilized the surfaces of leaves using 2% sodium hypochlorite solution for 30 second and rinsed three times in sterile distilled water. The leaves were cut and placed in Petri dishes lined with 4 layers of sterilized and moisten filter papers. The leaves were sprayed with spore suspensions of each pathogen isolate and incubated at 25^{o}C until typical symptoms of wilt and leaf spot were observed. The leaves sprayed with sterilized distilled water were used as a control. The causative agents on the diseased leaves parts were re-isolated on PDA media. The characteristics of the re-isolated fungal pathogens were compared with that of the original parent culture morphologically and microscopically.

3.9 Effect of *Trichoderma* metabolites on sesame seed germination

The effect of the culture filtrates of each *Trichoderma* isolate on the sesame seed germination was investigated. The inoculum preparation was carried out on potato dextrose broth medium. For inoculation with *Trichoderma* isolate, 10 % of spore suspension at a concentration of 10^{5} spore mL^{-1} was used. After incubation under shaking at 150 rpm at 25°C for 6 days, the culture broth was filtered using a filter paper. The filtrate was centrifuged at 5000 rpm for 15 minutes the supernatant was collected and the pellets were discarded. In this respect, sesame seeds were soaked for 24 h in each of the prepared *Trichoderma* filtrates. Untreated seeds served as a control. The seeds were dried for 1hr. under a Laminar flow cabinet. For each

treatment, 960 seeds were plated (40 seed/plate) on wet blotters and then incubated at 25°C for one week. Three replicates were used for each treatment. After which, the germination percent was calculated.

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seed}}{\text{Total number of seed}} \times 100$$

3.10. *In vitro* evaluation of antagonistic activity of *Trichoderma* isolates

The rapid growth of *Trichoderma* isolates against *Fusarium* and *Alternaria* isolates were evaluated using dual confrontation techniques (Afrasa Mulatu *et al.*, 2013; Syed *et al.*, 2015; Verma *et al.*, 2007; Weigle *et al.*, 2005) with slight modifications such as temperature, incubation period and pH. A mycelial agar plug (5 mm diameter) of each *Trichoderma* isolates taken from the edge of actively growing 7 days-old culture was paired against the same sized mycelia disc of the test pathogens at equal distances opposite to each other in 90 mm diameter Petri plates containing 20 ml PDA. The PDA plates inoculated only with *Trichoderma* isolates or *Fusarium* and *Alternaria* isolates served as control. There were six dual-cultures with three replicates for each *Trichoderma* and *Fusarium* isolates and *Trichoderma* and *Alternaria* isolates combinations. The dual cultures that were tested each other incubated at 25^{o}C for 10 days. The growth data of the pathogen isolates in both test and control experiments were recorded according to the method developed by Dennis and Webster, (1971) and Pakdaman *et al.* (2013). The percent inhibition of radial growth (PIRG) was computed as follows:

$$\text{PIRG} = \frac{(R1-R2) \times 100}{R1}$$

Where R1 = radial growth of pathogen in control. R2 = radial growth of pathogen in dual confrontation experiments with antagonists.

The antagonistic effect of *Trichoderma* isolates were assessed in semi-quantitative means, according to Rita and Tricita, (2004): >75 PIRG indicating very high antagonistic activity, 61–75 PIRG indicating high antagonistic activity, 51–61 PIRG, indicating moderate antagonistic activity, <50 PIRG, indicating low antagonistic activity, and 0 indicating no activity. A clear zone of inhibition (CZI) was also determined by measuring the clearance

between the colony margins of the *Fusarium* and *Alternaria* isolates and *Trichoderma* isolates.

3.11. Characterization of different bio control mechanisms of *Trichoderma* isolates

3.11.1. Effect of volatile metabolites of bio agents

Selected *Trichoderma* isolates based on the mycelium inhibition assay against sesame fungal pathogens isolates were evaluated for the production of volatile inhibitory substances under *in vitro* conditions following the modified methods of Dennis and Webster (1971a). Five-millimeter disc of *Trichoderma* colony was inoculated centrally in Petri plates containing PDA medium in triplicates. The Petri plates were sealed at the edges and incubated, at 25ºC. After 24 hr, the test pathogen was inoculated on fresh PDA and the lids of the Petri plates inoculated with antagonist were replaced by the pathogen on PDA. The plates were fixed with parafilm and incubated for another 6 days. The control plates were inoculated with pathogen alone (Dubey *et al.*, 2007). The radius of the test pathogen isolates was recorded and the percentage inhibition of radial growth (PIRG) was determined after ten days of incubation by using the same formula as described under dual culture plate testing (Section 3.10).

3.11.2. Effect of non-volatile metabolites of bio agents

The production of non-volatile substances by the *Trichoderma* isolates against the test pathogens were studied using the method described by Dennis and Webster (1971a); Dubey *et al.*(2007). *Trichoderma* isolates were inoculated in 100 ml sterilized potato dextrose broth (PDB) in 250 ml conical flasks and incubated at 25ºC on a rotatory shaker set, at 100 rpm for 15 days. The control flasks were not inoculated with any of the culture. The liquid culture was filtered through biological membrane filter paper (0.45µm) for removing mycelia mats (Aneja *et al.*, 2005). The filtrate was added to molten PDA medium to obtain a final concentration of 10% (v/v). Heat sterilized filtrate (10 ml) was mixed with 90 ml PDA, poured in Petri plates and 5 mm diameter culture disc of test pathogen isolates was inoculated at the center and incubated at 28ºC for 10 days. There were three times replicated of each treatment. The observation was taken and the mycelial growth inhibition percent was recorded and calculated in relation to the growth of the controls. The radius of the test pathogen isolates was recorded and the percentage inhibition of radial growth (PIRG) was determined after ten days of incubation by using the same formula as described in dual culture plate testing (Section 3.10).

3.12. Statistical analyses

Statistical analysis was performed using completely randomized analyses of variances (ANOVA) SPSS version 25 that was used to compare the biocontrol efficacy of *Trichoderma* isolates and means separated by Fisher's protected least significant difference. The significance of effects of *Trichoderma* on growth characteristics was determined by the magnitude of the F value ($p < 0.05$).

4. RESULTS

4.1. Isolation and Morphological identification of sesame fungal pathogens

It is clearly indicated from this study that out of 45 samples collected only 12 fungal isolates were isolated (Table 2). Out of 12 fungal isolates only five isolates of *Alternaria,* six isolates of *Fusarium* and one isolate of *Cercospora* were isolated from sesame seeds, leaves and soils collected from Wolkait district. The fungal pathogen isolates were characterized depending on their morphological and microscopic characters reported in various identification manuals and categorized into four genera (Table 2). These isolates were cultured to see their cultural characteristics: all *Alternaria* isolates developed loose, cottony and grayish-green to olive brown colonies on PDA after incubation at 25ºC for 7 days in the dark. Isolates were grouped morphologically, based on well-established features of *Fusarium* and *Alternaria* isolates, focusing on colony characteristics and conidial structure. It was observed that the majority of isolates were obtained from LalayMayhumer followed by Bet-Mulu and Kalema (Table 2). Quantitatively two isolate groups (AUA-1, AUF-5) were found most frequently and selected for further study (Table 2).

Table 2. List of fungal genera isolated from infected Leaves, Soils and seeds of sesame plants

S/N	Isolate Designation	Identified genera	Substratum	Location
1	AUA-1	*Alternaria* species	Leaf, seed	Bet-Mulu, Koratit
2	AUA-2	*Alternaria* species	Seed, soil	LalayMayhumer
3	AUA-3	*Alternaria* species	Seed	LalayMayhumer
4	AUA-4	*Alternaria* species	Soil	Kalema
5	AUF-5	*Alternaria* species	Soil	LalayMayhumer, Kalema
6	AUF-6	*Fusarium* species	Seed	LalayMayhumer
7	AUF-7	*Fusarium* species.	Leaf, soil	Kalema, Bet-Mulu
8	AUF-8	*Fusarium* species	Seed	Bet-Mulu, Koratit
9	AUP-9	*Fusarium* species	Leaf, seed	Bet-Mulu
10	AUP-10	*Fusarium* species.	Leaf	Kalema, Bet-Mulu
11	AUP-11	*Fusarium* species	Leaf	koratit
12	AUC-12	*Cercospora* species	Leaf	Kalema, Bet-Mulu

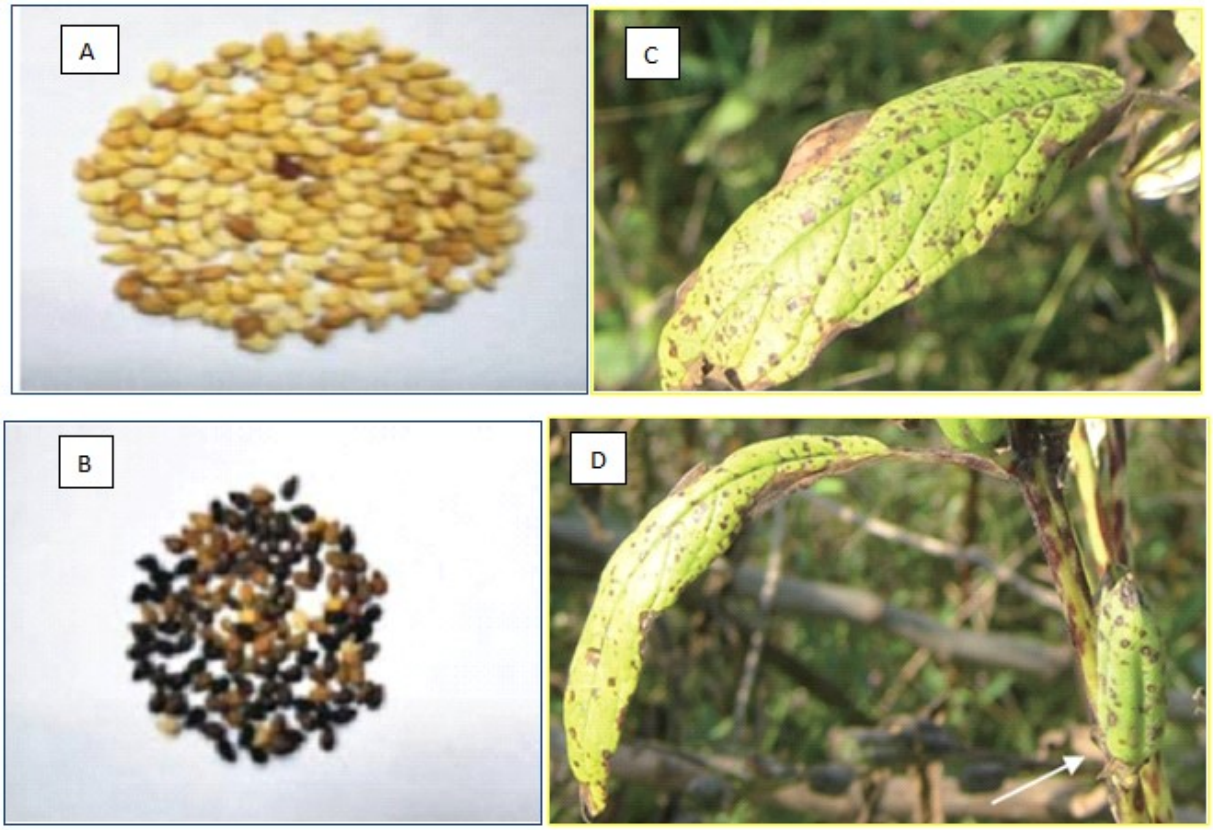


Fig. 3. (A) Healthy sesame seed, (B) Infected Sesame seed, (C) Typical leaf blight symptoms on sesame leaves in the field and (D) pods (arrow) during the sampling in Wolkait district.

4.2. Pathogenicity test

After seven days inoculation with a spore suspension of the *Alternaria* (AUA1) and *Fusarium* isolates (AUF5), brown lesions symptoms surrounded by yellow haloes began to develop on sesame leaves (Fig. 3). Disease symptoms gradually spread from leaf margins to the mid veins similar to symptoms observed in the sesame fields during the sample collection (Fig.3). At a later stage, blight extended to the center of the leaves. The symptoms of leaf blight found on the leaves were initially round to irregular, brown colored necrotic spots with concentric zonation demarcated with brown lines inside the spots on the upper surface (Fig.3). In severe infection, several spots would coalesce together involving a major portion of the leaf blade and the affected leaves would dry and usually drop off. The symptoms of *Fusarium* wilt were shown with yellow and dropped leaves, often starting on one side, and stunting of the plant. Disease symptoms initiated at the bottom of the stem and progressed upwards, causing the leaves to wilt, wither, and die. Similarly, the infection of seeds and leaves of sesame by *Alternaria* and *Fusarium* isolates were observed on filter papers in the Petri dishes (Fig. 4).

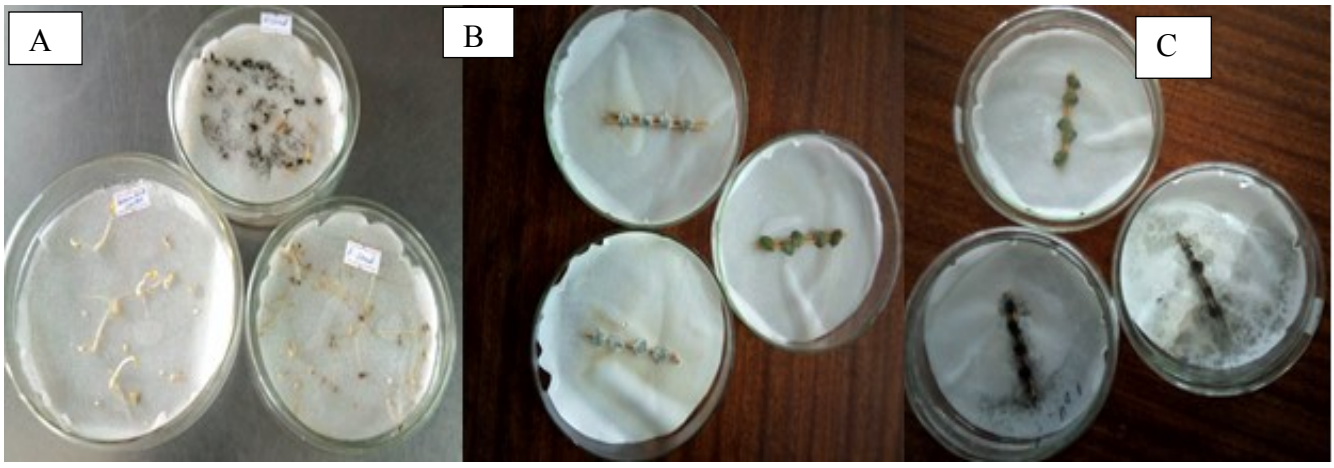


Fig. 4. Pathogenicity test: (A) Pathogenicity test of *Alternaria* and *Fusarium* isolates on seed, (B). Pathogenicity test of *Fusarium* isolate on detached sesame leaf (C). Pathogenicity test of *Alternaria* isolate on detached sesame leaf.

4.3. Cultural and microscopic characteristics of sesame fungal pathogens

The sesame pathogens were culturally and microscopically characterized based on the size, color and shape of mycelia, and conidia (Table 3 and Fig. 5).

Table 3. Culture and microscopic characteristics of pathogen isolates (400x)

Selected sesame pathogens		**Morphological and cultural characteristics**			
	Isolate Designation	Spore size (µm)	Colony Color (front)	Conidia shape	Mycelial shape
Alternaria isolate	AUA1	80	Blue black	Oval	Raised, no rings
Fusarium isolate	AUF5	90	Creamy	Falcate	Raised with rings

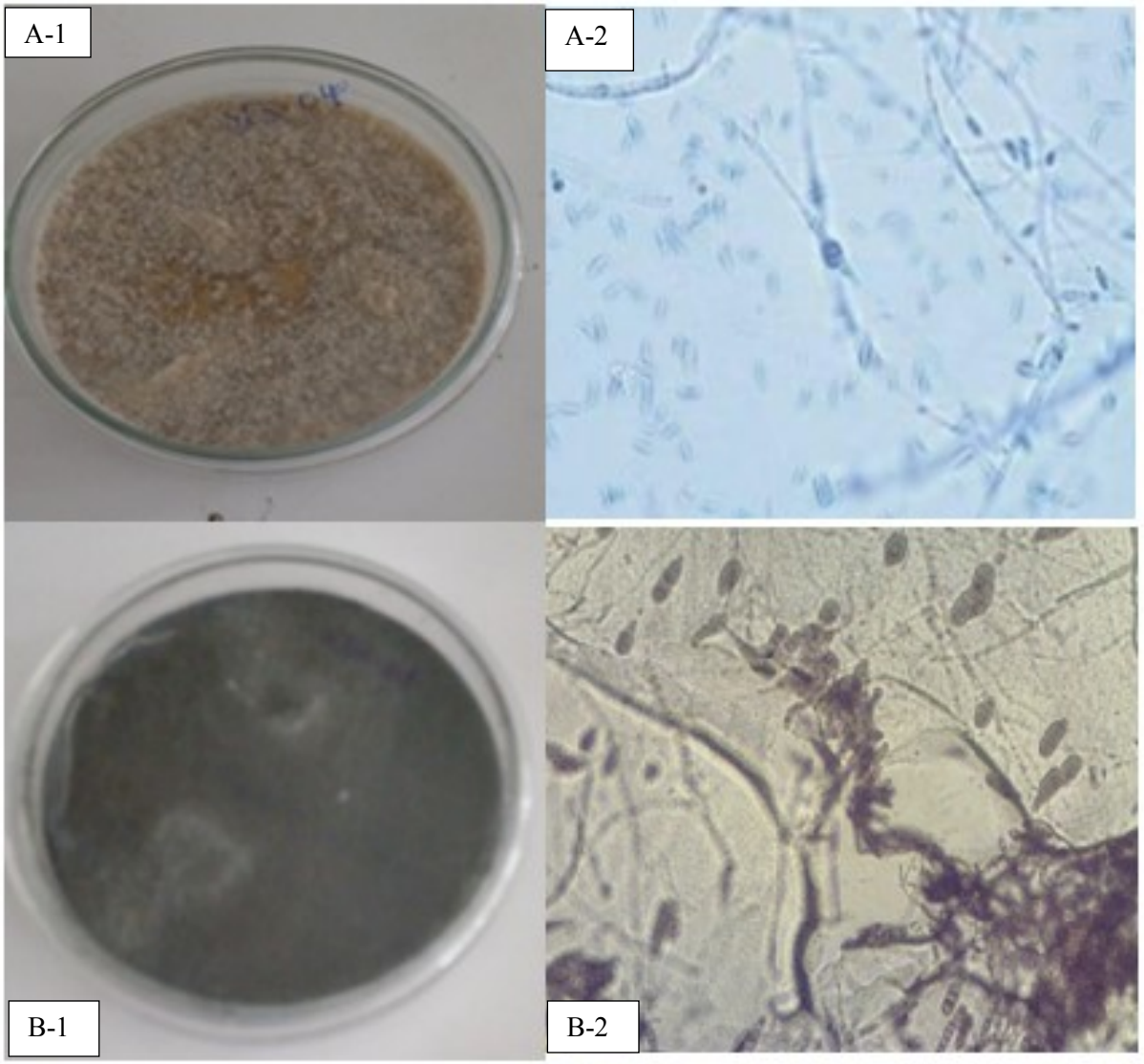


Fig. 5. Colony morphology of selected sesame fungal pathogens: *Fusarium* isolate (AUF5): pure culture (A1) and Chlamydospores (A2). ***Alternaria*** **isolate** (AUA1): Pure culture (B1) and Chlamydospores (B2).

4.4. Cultural and morphological characteristics of *Trichoderma* isolate

The isolates were found to form colonies with white mycelia, becoming green when conidia and conidiophores formed (Table 4). Conidia formed densely over the center and undulating concentric rings toward the edge (Fig.6). Observation through the bottom of Petri dishes showed production of yellowish/cream-white pigmentations by some isolates at an early age (Fig.6). These colors either remained with time or changed into purple or black (Fig.6).

Table 4. Morphological and Cultural characteristics of *Trichoderma* isolate

Isolates	Cultural characteristics of the colony			
	Spore size (µm)	Colony color (Back)	Conidia shape	Mycelial shape
AUT-11	4	Bright green	Oval	Raised, no rings
AUT-12	4	Bright green	Grape	Raised with rings
AUT-32	6	Yellow	Oval	Effused & light
AUT-33	6	Yellow	Oval	Raised with rings
AUT-97	8	Green	Oval	Raised, no rings
AUT-131	8	Yellowish green	Oval	Raised, no rings
AUT-158	4	Deep green	Oval small and numerous	Effused, no rings

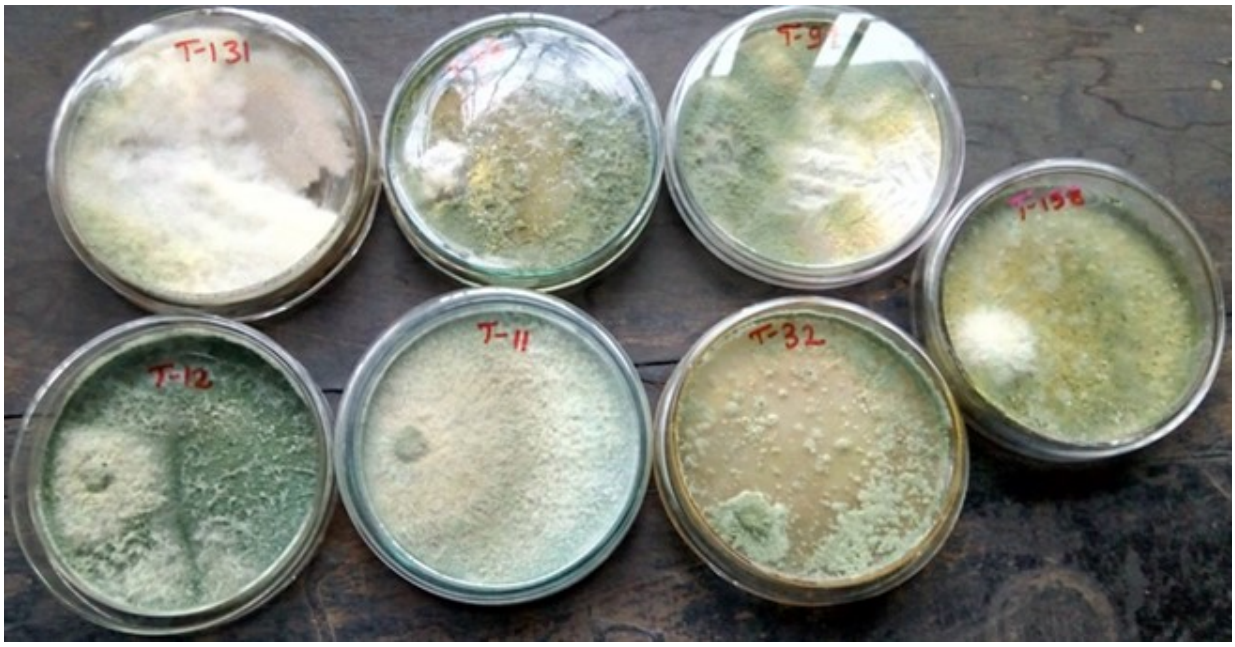


Fig. 6. Pure culture of seven *Trichoderma* isolates

4.5. The effect of temperature on the growth of Trichoderma isolates

Almost all *Trichoderma* isolates grown on the PDA plates, at 25ºC showed full growth within 4 days because of their higher growth rates (Table 5). But there were slight variations among some of the fungal isolates. Accordingly, isolates AUT-97, AUT-131 and AUT-158 were significantly ($P \leq 0.05$) expressed the highest growth rate. While the slowest growth rates were expressed by isolate AUT-11. The finding revealed that 25ºC temperature was the optimum temperature for mycelial growth in batch culture (Table 5). In contrast to the above-mentioned temperature, isolate AUT-32 and AUT-97 grown on PDA plates showed confluent growth within 5 days at 37ºC, whereas the other isolates grew up to 1 cm on the Petri dish (9 cm). On the other hand, all the isolates couldn't grow, at 4ºC. Therefore, the improvement of stress tolerance in *Trichoderma* isolates could result in increasing their efficacy against plant pathogenic fungal isolates even under unfavorable environmental conditions. It is clearly evident from this experiment that temperature is very important and relevant for the growth and infection process of the fungal pathogen isolates.

Table 5. The average colony diameters of each *Trichoderma* isolates grown on PDA plates, at 25ºC

S. No	**Isolate code**	**Average colony diameter (cm)**			
		Day 1	**Day 2**	**Day 3**	**Day 4**
1	AUT-11	2.8 ± 0.15	4.5 ± 0.07	6.8 ± 0.12	8.5 ± 0.08
2	AUT-12	3.3 ± 0.01	6.2 ± 0.09	8.6 ± 0.03	9.00
3	AUT-32	2.9 ± 0.15	6.3 ± 0.14	8.5 ± 0.12	9.00
4	AUT-33	3.2 ± 0.05	7.2 ± 0.05	8.2 ± 0.13	9.00
5	AUT-97	3.6 ± 0.15	8.1 ± 0.05	9.00	9.00
6	AUT-131	3.5 ± 0.12	7.4 ± 0.06	9.00	9.00
7	AUT-158	4.4 ± 0.06	8.0 ± 0.03	9.00	9.00

4.6. Biomass production and spore yield determination of Trichoderma isolates at different pH value

The effect of pH on the mycelia growth and spore yield by *Trichoderma* isolates after 10 days in broth medium is shown in (Fig. 7 and 8). The finding revealed that pH 7.5 supported the maximum mycelial growth of 0.32, 0.28 and 0.27g/ml produced by isolates AUT-97, AUT-32 and AUT-131 in batch culture, respectively, while the lowest mycelial growth of 0.08g/ml was recorded, at pH 4.5 (Fig.7). The optimum pH for the mycelial growth of *Trichoderma* isolates was 4.5-7.5 in broth medium. The biomass production after 10 days, i.e., at the end of the experiment ranged from 0.08-0.32g in all treatments. With increasing time, all the isolates showed a significant ($P<0.05$) increase in biomass at all pH levels.

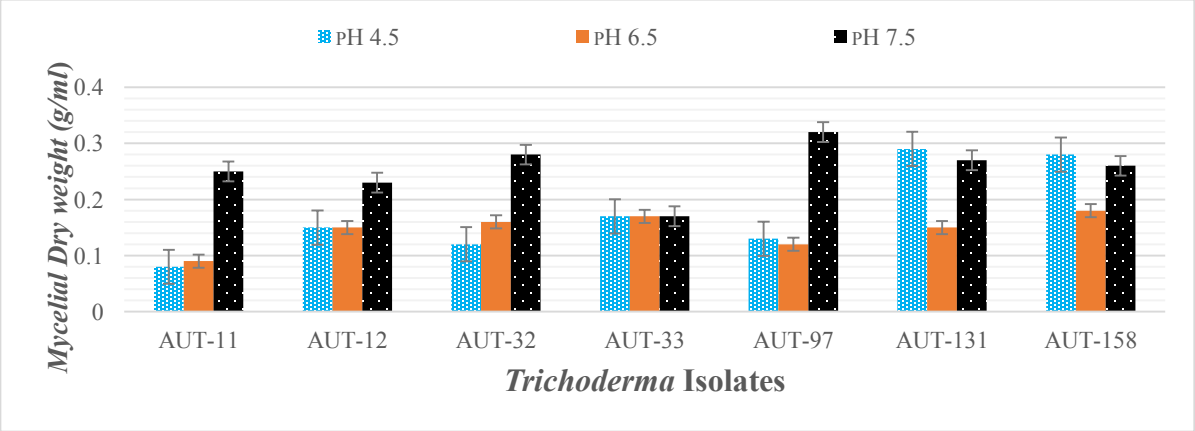


Fig.7. Influence of pH on the mycelial growth of *Trichoderma* isolates after 10 days in broth

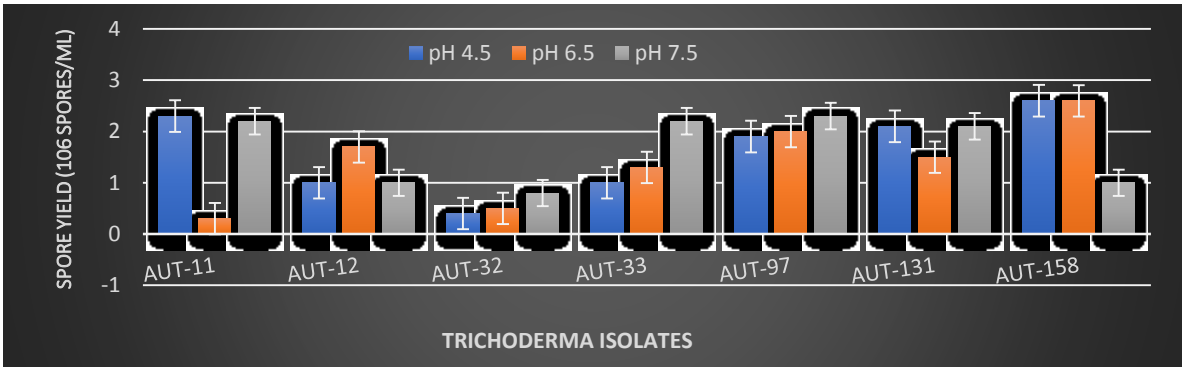


Fig.8. The effect of pH on the spore yield of *Trichoderma* isolates

4.7. Effect of Trichoderma metabolites on sesame seed germination

Results showed that all the *Trichoderma* isolates were found effective to enhance the germination percentage compared to control (Fig.9). However, among the seven *Trichoderma* isolates, AUT-12 (93 %), AUT-158 (91.6 %) and AUT-97 (90.8%) exhibited a significant (P≤0.05) enhancement of germination percentage in sesame seeds in the laboratory (*in vitro*) conditions followed by AUT-33 (85.8%), AUT-11 (60%), AUT-32 (64.2%) and AUT-131 (57.5 %), (Fig. 9), while control treated with water significantly decreased these values (55 %).

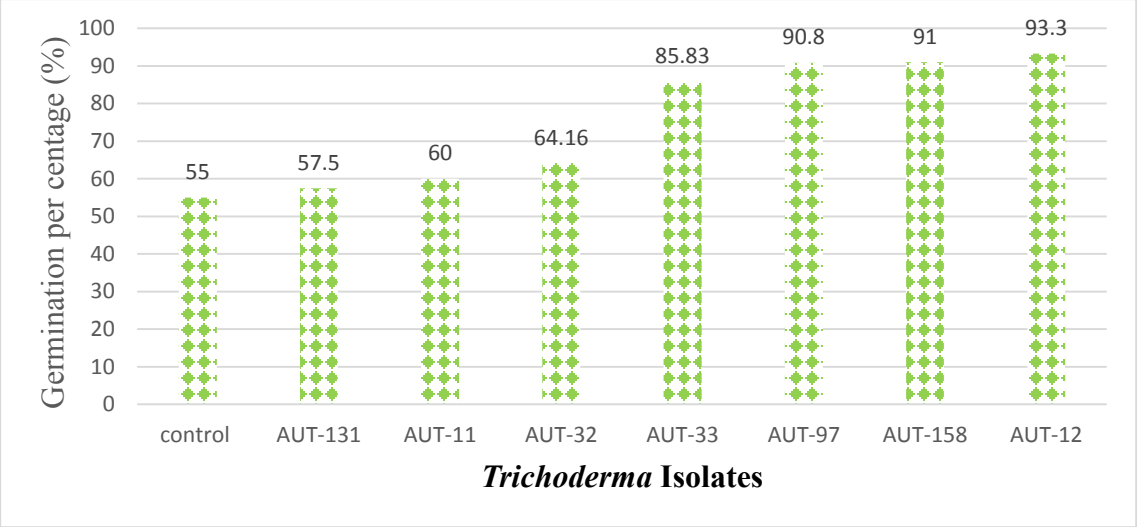


Fig. 9. The effect of *Trichoderma* isolates on seed germination

4.8. Antagonistic activities using a dual culture test

Using a dual confrontation assay, it was found that all the *Trichoderma* isolates exhibited antifungal activity against the test pathogens of sesame crop (Table 6). By comparing and contrasting the data obtained from the dual confrontation assays, it was found that all the 7 *Trichoderma* isolates inhibited the mycelial growth of AUA1 isolate within the range of 69.25-76.42% against AUA1 and 66.26-79.49% against AUF5 ($P < 0.05$). Often, *Trichoderma* isolates grew within 6 days and invade both the test pathogens colony tested (Table 6 and Fig.10). The highest-level inhibition of AUA1 isolate was shown by AUT-97 (76.42%) and the lowest level was recorded for AUT-33 (69.25%). Furthermore, it was observed that the highest-level of inhibition of AUF5 isolate was shown by AUT-131 (79.49%) and the lowest level was recorded for AUT-33 (66.26%).

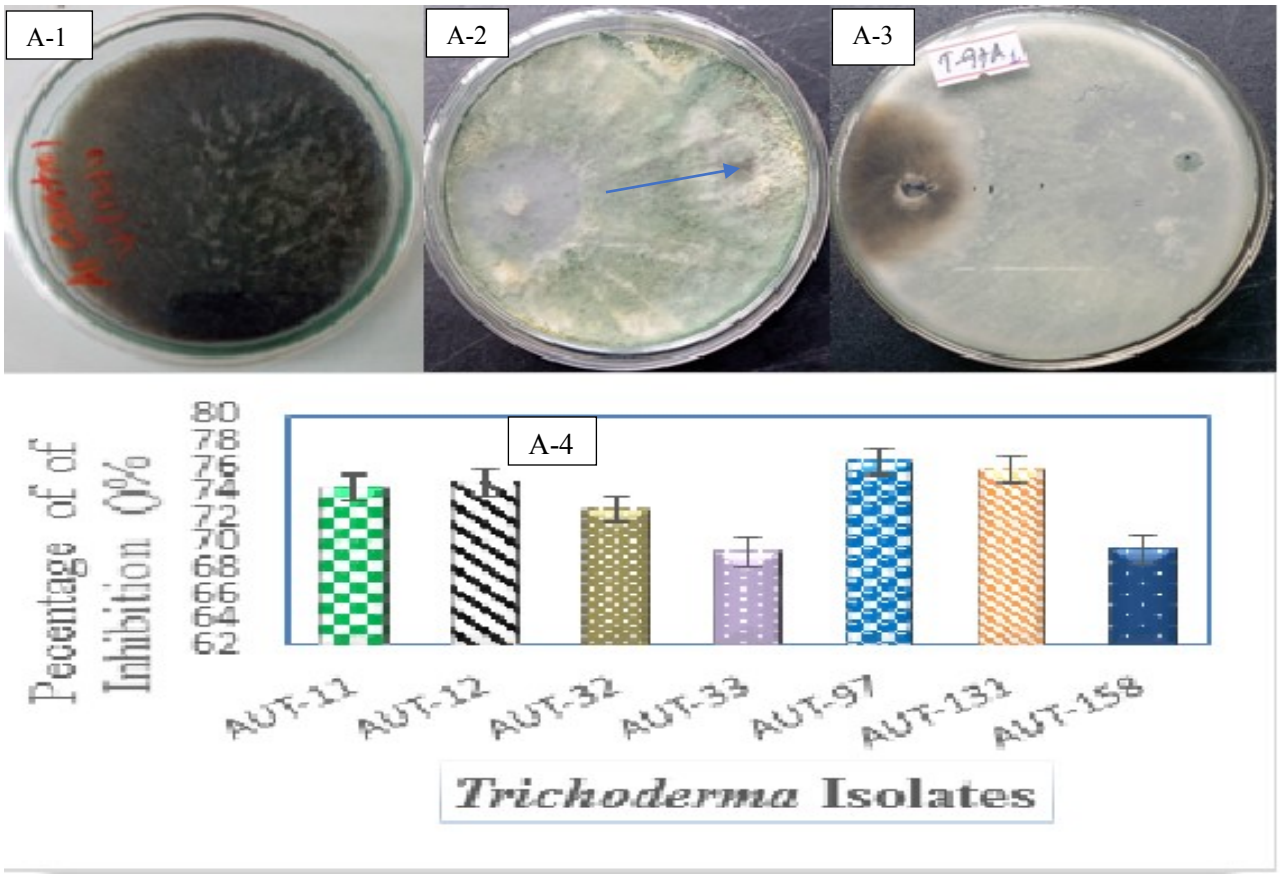


Fig. 10. Typical dual confrontation assay that was used to quantify the interactions of 7 *Trichoderma* isolates with pathogen of *Alternaria* isolates. Control plate with *Alternaria* isolates (A1), reverse colony dual confrontation of *Alternaria* isolates (indicated by arrows) with *Trichoderma* (A2), front surface colony dual confrontation of *Alternaria* isolates (A3), mean relatively sensitive of *Fusarium* and *Alternaria* isolates to *Trichoderma* isolates (A4).

Table 6. *In vitro* evaluation of *Trichoderma* isolates against AUA1 and AUF5 isolates by dual confrontation culture technique, volatile and non- volatile compounds

Trichoderma isolates	*Alternaria* isolate (AUA1)			*Fusarium* isolate (AUF5)			Scale of antagonistic activity
	Dual culture	Volatile compound	Non-volatile compound	Dual culture	Volatile compound	Non-volatile compound	
AUT-11	74.32^{ab}± 4.06	77.9^{ab}±6.1	88.77^{a} ±7.7	76.12^{ab}± 6.20	76.2^{ab}±6.7	90.12^{a} ±6.04	+ + + +
AUT-12	74.82^{a}± 4.20	71.47^{b}± 10.2	82.05^{ab} ± 13.00	71.07^{cd}± 6.50	72.8^{b} ± 12.6	90.12^{a} ± 6.18	+ + +
AUT-32	72.71^{ab}± 4.82	66.5^{b}± 14.65	16.38^{d} ± 17.01	68.78^{cd}± 7.73	66.5^{b} ± 14.6	55.79^{c} ± 14.99	+ +
AUT-33	69.25^{b}± 4.83	65.8^{b} ± 20.52	25.25^{d} ± 21.49	66.26^{d} ± 9.32	65.8^{b} ± 20.5	58.86^{c} ± 16.56	+ +
AUT-97	76.42^{a} ±3.68	93.65^{a} ± 2.4	87.32^{a} ± 7.52	71.86^{cd}± 6.86	93.65^{a} ± 2.4	87.33^{a} ± 7.52	+ + + +
AUT-131	75.82^{a} ± 4.81	92.16^{a}± 3.8	71.59^{bc} ± 11.61	79.49^{a}± 3.26	92.2^{a} ± 3.8	71.59^{b} ± 11.61	+ + + +
AUT-158	69.50^{b} ± 5.12	44.17^{c} ± 8.61	65.73^{c} ± 4.21	72.67^{bc}± 5.79	44.2^{c} ± 8.69	65.74^{b} ± 4.21	+ +
Total Mean ± Sd	73.3 ± 5.08		59.61 ± 32.43	69.04 ± 16.91	69.72 ± 24.3	70.85 ± 22.88	-

AUA1= Addis Ababa University *Alternaria* isolate 1 and **AUF5**= Addis Ababa University *Fusarium* isolate 5

Descriptive assessment of the antagonistic activity as previously described by Rita and Tricita, (2004) was scaled as follows:

++++ = very high antagonistic activity (> 75 PIRG). +++ = high antagonistic activity (61–75 PIRG).

++ = moderate antagonistic activity (51–60 PIRG). + = low antagonistic activity (< 50 PIRG) and - = no antagonistic activity.

Different alphabets depicted in superscript in the columns indicate mean treatments that are significantly different according to Tukey's HSD post-hoc test at $P < 0.05$. Each value is an average of 9 replicate samples ± Standard error.

4.9. *In vitro* characterization of different bio control mechanisms of *Trichoderma* isolates

4.9.1. Effect of volatile metabolites

The volatile compounds released by *Trichoderma* isolates have also exerted inhibitory effect on the growth of the selected pathogens. The results of the test to produce diffusible inhibitors are also presented in (Table 6**)**. Isolate AUT-97 and AUT-131 showed highest inhibition effect on the percent mycelia growth of both AUA1 (92.16%-93.65%) and AUF5 (92.2% - 93.65%), respectively. Least per cent mycelium growth inhibitions of AUA1 and AUF5 were occurred in the interaction with AUT-158 (44.17%), and AUT-158 (44.2%), respectively. Most of the isolates showed percent mycelium inhibition values ranged between 65.8 - 77.9% against the test pathogen isolates. The remaining isolates were poor producers of diffusible metabolites. The interaction between test pathogens and *Trichoderma* isolates were determined and illustrated (Table 6).

4.9.2. Effect of non-volatile/diffusible metabolites of bio agents

In this experiment with the non-volatile metabolites results obtained demonstrated significant differences in inhibitory effects of the non-volatile metabolites obtained from the different isolates of *Trichoderma* species. In the present study, the most efficient *Trichoderma* isolates overall was both AUT-11 and AUT-12, which inhibited the two phytopathogenic fungal isolates (AUA1, and AUF5) growth by diffusible assays (90.12%). The remaining *Trichoderma* isolates displayed a range of antagonist activity from 16.38 % to 88.77 % (Table 6).

5. DISCUSSCUSION

This study clearly demonstrated that *Alternaria* and *Fusarium* isolates are present in a high proportion of sesame seeds collected from various regions in Wolkait district that suggested the existence of inoculum to initiate disease outbreaks under favorable conditions to pathogen growth and development. Species delineation within the genus *Alternaria* and *Fusarium* requires careful attention in order to determine the range of isolates causing diseases on a sesame host. Among the major diseases that affect sesame is *Fusarium* wilt and *Alternaria* leaf blight, which are caused by *Fusarium oxysporum sesami* and *Alternaria sesami. Alternaria* leaf spot (ALS) of sesame caused by the seed-borne fungus *Alternaria sesami* (Kaw.) Mohanty and Behera is of worldwide rich (Leppik and Sowell, 1964). In the present study, 12 fungal isolates were isolated, among them 5 of the isolates of *Alternaria,* 6 isolates of *Fusarium* and 1 isolate of *Cercospora* were recorded from sesame seeds, leaves and soils collected from Wolkait district (Table 2). It was observed that the majority of isolates were obtained from LalayMayhumer followed by Bet-Mulu and Kalema. Similarly, Shekharappa and Patil, (2001) has observed symptoms on infected plants included lesions with concentric rings on leaves that often coalesce to form large blighted leaf areas.

On the other hand, *Fusarium* wilts caused by *Fusarium oxysporum f. spp. Sesami* (FOS) is a devastating disease infecting sesame crop right from seedling to maturity resulting in crop losses in varying degrees depending on the severity of infection. It has been reported as the most important soil borne fungal disease in which the water-conducting (xylem) vessels become blocked, so that the plant wilts and finally dies (Zhang *et al.*, 2019). The symptoms of leaf blight found on the leaves were initially round to irregular, brown colored necrotic spots with concentric zonation demarcated with brown lines inside the spots on the upper surface. The symptoms of the disease in the present study were found to be similar to the typical symptoms of the disease described earlier Ashri, (1998). In severe infection, several spots would coalesce together by involving a major portion of the leaf blade and the affected leaves would dry and usually drop off. The disease was observed to occur at all stages of plant growth (Ojiambo *et al.*, 2003).

Aggregate species of *Trichoderma* can be differentiated on the basis of macroscopic (colour of conidia, sporulation patterns and density) and microscopic (structure and arrangement of phialides, conidial size and shape) features. In the present study it was observed under light microscope (400x) that all the seven *Trichoderma* isolates had an oval-shaped spore, which were almost equal in size except isolate AUT-11, AUT-12 and AUT-158 which had numerous and small-sized spores. Cultural characteristics comprising growth rate, colony colour and colony appearance were regarded as taxonomically useful characteristics for *Trichoderma* species (Samuels *et al*., 2002). Morphological characterization was conventionally used in the identification of *Trichoderma* species, and it remains as a potential method to identify *Trichoderma* species (Anees *et al*., 2010; Gams and Bissett, 2002; Samuels *et al*., 2002).

Taxonomic knowledge on *Trichoderma* isolates is important for identification and characterization of potential biocontrol species to avoid risk of introducing an unknown fungal species into the rhizosphere of a given ecosystem. A combination of morphological and molecular methods is desirable for the reliable and accurate identification of *Trichoderma* species. The few morphological characteristics with limited variation in *Trichoderma* species may lead to an overlap and wrong identification of the species (Bunbury-Blanchette and Walker, 2019).

The growth and conidiation of *Trichoderma* were influenced by some known environmental factors that include the pH of the medium, temperature, physical injury to the mycelium and the presence of fungal-derived volatile organic compounds (Steyaert *et al.,* 2010a). In this study, different pH and temperature conditions affected the mycelial growth and conidiation of *Trichoderma* isolates. The study tested the pH range favored the mycelial growth, spore yield and conidiation of *Trichoderma* isolates with different values. It has been demonstrated that *Trichoderma* isolates were active under a wider range of pH (Kredics *et al.,* 2003) and from our study, it can be deduced that *Trichoderma* isolates grew and sporulates maximally at optimum pH.

Similar to other physiological parameters, pH plays a significant role in the growth and sporulation. Analysis revealed that pH 7.5 supported the maximum mycelial growth produced by isolates AUT-97, AUT-32 and AUT-131 in batch culture, respectively. Temperature and pH are two key parameters to manipulate growth, sporulation and saprophytic ability as well

as production of volatile and non-volatile metabolites, involved in nutrition, competition, mycoparasitism and extra cellular enzymes that disintegrate cell wall of fungi. Therefore, it is important to collect information about the effects of pH and temperature on the mycelial growth. It has been demonstrated that *Trichoderma* strains are active under a wider range of pH (Kredics *et al*., 2003). The optimum temperature for growth differs among the *Trichoderma* isolates; although most *Trichoderma* strains are mesophilic (Kredics *et al*., 2003). The results obtained from the present study also support these hypotheses. The temperature could affect their metabolic activity, especially the production of volatile antibiotics and enzymes (Dennis and Webster, 1971b). Though temperature plays an important role in the growth of organisms, at an elevated level, it damages the organisms by denaturing enzymes, transport carriers, integrity of cell membranes (Prescott *et al.,* 2002).
In this study, seven *Trichoderma* isolates gave early germination as well as highest germination percentage which, have also been reported by Hanson, (2000) in different plants.

Strong antagonism by *Trichoderma* species against a range of soil borne plant pathogens has been reported (Bhagat and Pan., 2008). The present study determined the potential antagonistic variation of isolates of *Trichoderma* in the two-soil borne and foliar phytopathogens of *Fusarium* and *Alternaria* isolates. The antagonistic capabilities of seven *Trichoderma* isolate were assessed by the inhibition of *Fusarium* species *and Alternaria* species growth through the dual culture test and about 28.6% of the isolates showed the highest inhibition values greater than 75% (very high antagonistic), the rest of them 71.4% showed high inhibition values in the range of 66.26 -75% (Rita and Tricita, 2004). The results revealed that *Trichoderma* isolate (AUT-131) was found to effectively inhibit the radial mycelial growth of the pathogen (by 79.49%) compared to all other isolates. Negash Hailu (2007) has also reported that *Trichoderma* species against *F. xylarioides* did not show any clear zone, but they overgrew the pathogen and occupied all over the media and totally surrounded the fungus in a test. Similar results were reported by Solanki *et al*. (2011) in their antagonistic study of *Trichoderma* species against *Rhizoctonia solani*. Kotasthane *et al*. (2015) in their antagonistic study of *Trichoderma* species against two phytopathogens (*Sclerotium rolfsii* and *Rhizoctonia solani*) under *in vitro* confrontation assay reported that *T. viride* isolate T14 showed 100 % inhibition against *R. solani*. Akinbode and Ikotun (2010)

have shown antagonistic potentials of two *Trichoderma* species, in which *T. pseudokoningii* had better inhibition of the mycelia growth of *Colletotrichum destructive* than *T. harzianum*.

Seven *Trichoderma* isolates evaluated, performed well as bio control agents when tested individually with *Alternaria* and *Fusarium* isolates on culture media. The *in vitro* evaluation of dual culture technique exhibited that the mycelial growth of the pathogenic fungal isolates were suppressed by the production of volatile and nonvolatile compounds of *Trichoderma* isolates. Isolates, AUT-97 and AUT-131 showed the highest inhibition effect on the percent mycelia growth of both AUA1 (92.16%-93.65%) and AUF5 (92.2%-93.65%), respectively. Most of the isolates showed percent mycelium inhibition values between 65.8 - 77.9% against the test pathogen isolates. The remaining isolates were poor producers of diffusible metabolites. Vey *et al*. (2001) have reported that there are large varieties of volatile secondary metabolites produced by *Trichoderma* species which play an important role in controlling the plant pathogens (Bhagat *et al*., 2014).

In the present study, the most efficient *Trichoderma* isolates overall were both AUT-11 and AUT-12, which inhibited the two phytopathogenic (AUA1, and AUF5) growth by diffusible assays (90.12%). The *in vitro* confrontation assay has shown to be a useful and reliable method for identifying the biocontrol efficacy of *Trichoderma* strains (Hermosa *et al.*, 2012). *Trichoderma* species have been reported to produce a plethora of secondary metabolites possessing antimicrobial activity (Verma *et al.*, 2007). The ability of *Trichoderma* species to control *Fusarium* wilt was reported before in tomato (Devi, 2012; Hassan *et al*., 2014). The results of this study are in accordance with previous results which mentioned the high capability of micro-organisms to control sesame wilt disease (Ruocco *et al.*, 2015) and agree with previous studies that found application of *Trichoderma* species *is* very efficient to suppress *Fusarium oxysporum sesami* and can present an efficient method to control *Fusarium* wilt in sesame (Elewa *et al*., 2011). On the other hand the results revealed that the antagonists significantly reduced the growth of *Alternaria sesami* either by competition (over growing) or by antibiosis (exhibiting inhibition zones). The inhibition of mycelial growth of *Alternaria sesami* by *Trichoderma* isolates could be obviously attributed to several possibilities of existence of microbial interactions such as higher competitive ability, stimulation and antibiosis by these isolates over the test pathogen. The antagonism of

Trichoderma species against many fungi is mainly due to production of acetaldehyde compound (Dennis and Webster, 1971).

6. CONCLUSIONS AND RECOMMENDATION

6.1. Conclusion

It is clearly observed that from under an *in vitro* bioassay that the highest mean inhibitory effect against the growth of the fungal pathogen isolates were achieved by AUT-11, AUT-12 and AUT-97 isolates in dual culture. These isolates showed consistent results in volatile and non-volatile activity under *in vitro* condition against both of the two fungal pathogen isolates tested. The highest mean inhibitory effect on the growth of the test pathogen isolates were achieved by AUT-97 isolate (76.42%) against *Alternaria* species and AUT-131 isolate (79.49%) against *Fusarium* isolates restricting it almost completely in plates as compared to the control consisting of any of the two test pathogens growing alone. Therefore, *Trichoderma* isolates significantly ($p<0.05$) inhibit the radial growth of the test pathogen isolates.

6.2. Recommendations

From the outcome of this study, the following recommendations are forwarded.

- Evaluation of the potential *Trichoderma* isolates should be further studied under greenhouse and field conditions against *Fusarium* species and *Alternaria* isolates to determine their effectiveness in disease control.
- Evaluation of the effect of seven *Trichoderma* isolates on seed germination to be well studied under greenhouse and field conditions against *Fusarium* and *Alternaria* isolates in the future to determine their effectiveness.
- For exploitation of potential antagonistic *Trichoderma* isolates in soils which will be applied as biocontrol agent must be studied the effect of temperature, pH and other factors on their mycelial growth.

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APPENDECES

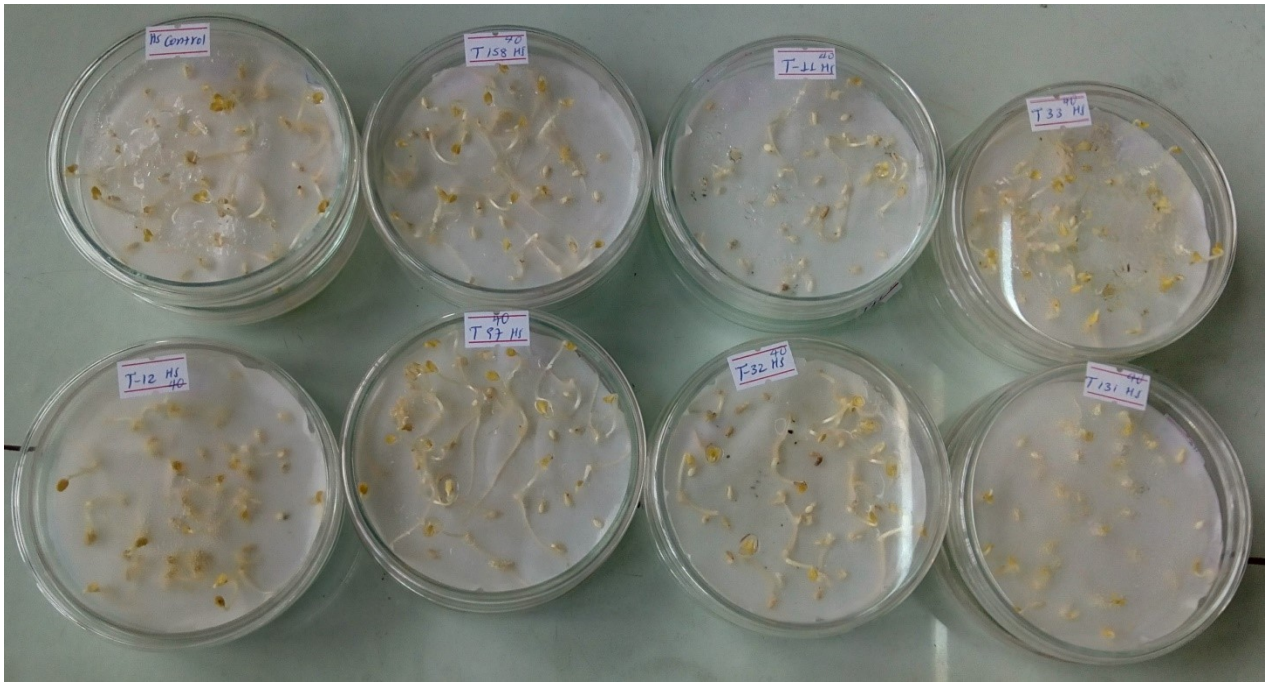
Appendix 1. Visual observations of leaf blight and *Fusarium* wilt of sesame

Typical symptoms of leaf blight of sesame

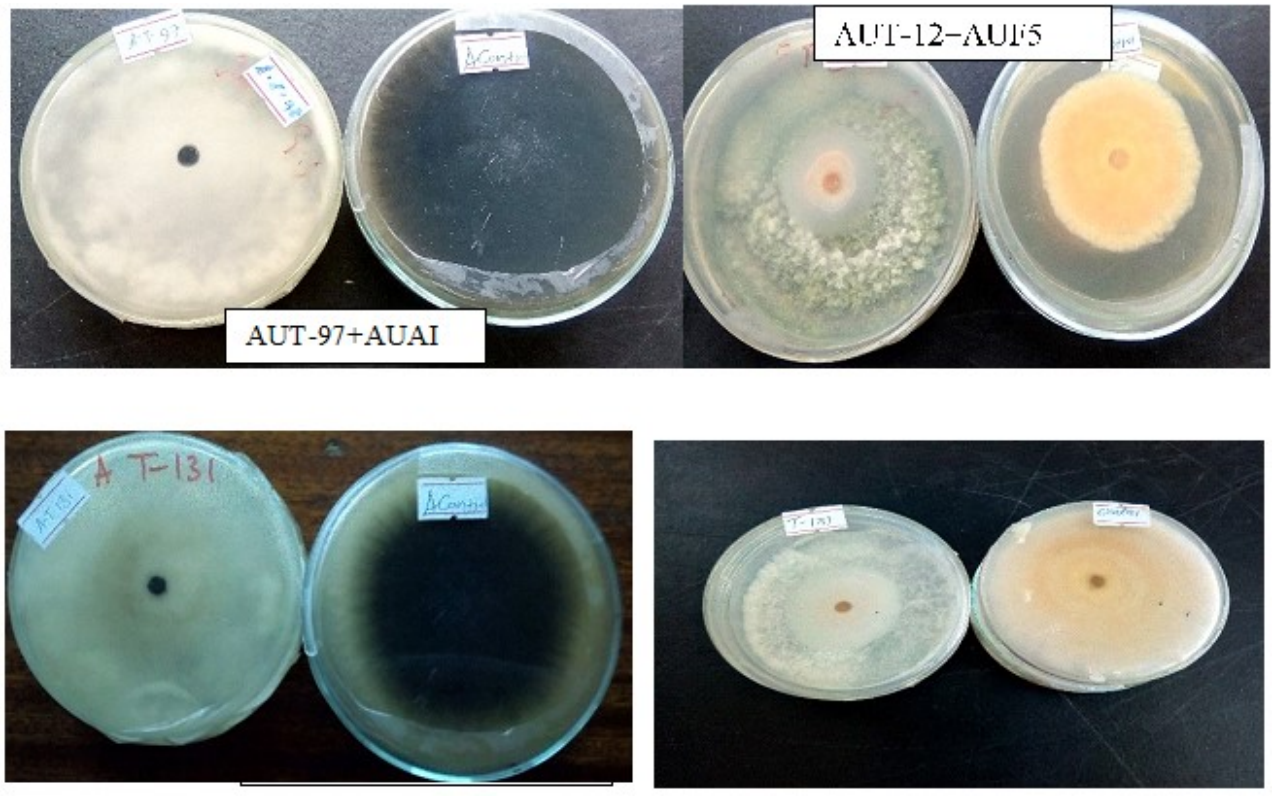
Typical symptoms of fusarium wilt of sesame

.

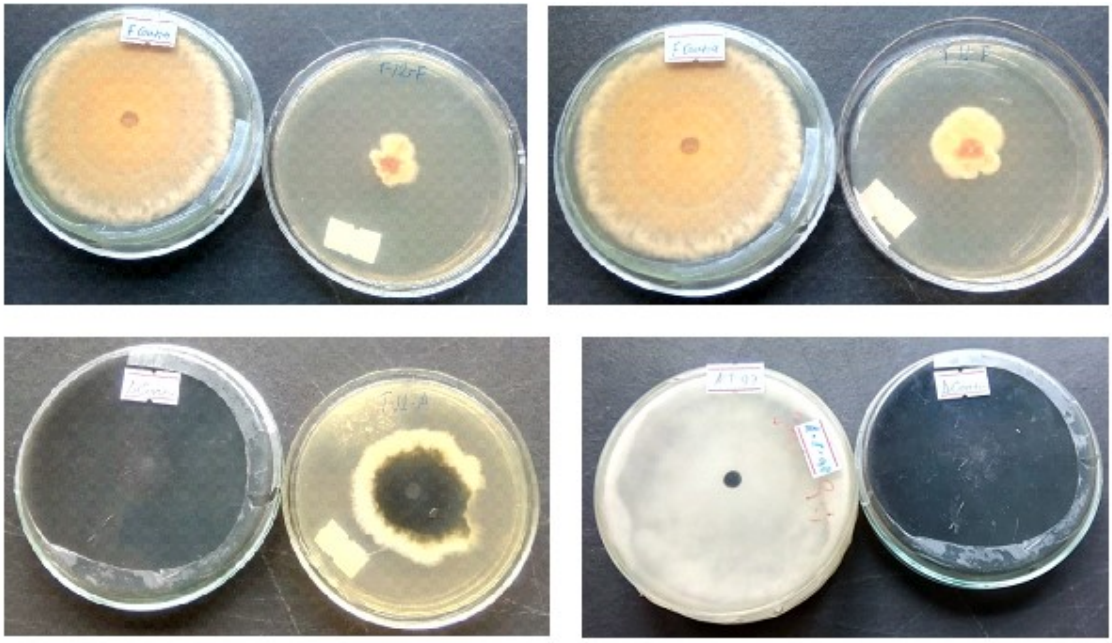
Appendix 2. Effects of *Trichoderma* isolates on seed germination of sesame



Appendix 3. Effects of volatile compounds of *Trichoderma* isolates against *Alternaria* and *Fusarium* species



Appendix 4. Effects of non-volatile compounds of *Trichoderma* isolates against *Alternaria* and *Fusarium* species



Appendix 5. Tukey's Post HOC tests for *Alternaria* isolate AUA1 under Dual culture test

Pair wise Comparisons

Dependent Variable: PRGI

(I) Trichoderma isolates	(J) Trichoderma isolates	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Control	T-11	-74.322*	2.081	.000	-81.274	-67.370
	T-12	-74.822*	2.081	.000	-81.774	-67.870
	T-131	-75.818*	2.081	.000	-82.770	-68.866
	T-158	-69.500*	2.081	.000	-76.452	-62.548
	T-32	-72.714*	2.081	.000	-79.667	-65.762
	T-33	-69.248*	2.081	.000	-76.200	-62.296
	T-97	-75.681*	2.123	.000	-82.777	-68.586
T-11	Control	74.322*	2.081	.000	67.370	81.274
	T-12	-.500	1.471	1.000	-5.416	4.416
	T-131	-1.496	1.471	1.000	-6.412	3.420
	T-158	4.822	1.471	.060	-.094	9.738
	T-32	1.607	1.471	1.000	-3.309	6.523
	T-33	5.074*	1.471	.037	.158	9.990
	T-97	-1.359	1.531	1.000	-6.476	3.757
T-12	Control	74.822*	2.081	.000	67.870	81.774
	T-11	.500	1.471	1.000	-4.416	5.416
	T-131	-.996	1.471	1.000	-5.912	3.920
	T-158	5.322*	1.471	.023	.406	10.238
	T-32	2.107	1.471	1.000	-2.809	7.023
	T-33	5.574*	1.471	.014	.658	10.490
	T-97	-.859	1.531	1.000	-5.976	4.257
T-131	Control	75.818*	2.081	.000	68.866	82.770
	T-11	1.496	1.471	1.000	-3.420	6.412
	T-12	.996	1.471	1.000	-3.920	5.912
	T-158	6.318*	1.471	.003	1.402	11.234
	T-32	3.104	1.471	1.000	-1.812	8.020
	T-33	6.570*	1.471	.002	1.654	11.486

	T-97	.137	1.531	1.000	-4.980	5.254
T-158	Control	69.500*	2.081	.000	62.548	76.452
	T-11	-4.822	1.471	.060	-9.738	.094
	T-12	-5.322*	1.471	.023	-10.238	-.406
	T-131	-6.318*	1.471	.003	-11.234	-1.402
	T-32	-3.214	1.471	.971	-8.130	1.701
	T-33	.252	1.471	1.000	-4.664	5.168
	T-97	-6.181*	1.531	.006	-11.298	-1.064
T-32	Control	72.714*	2.081	.000	65.762	79.667
	T-11	-1.607	1.471	1.000	-6.523	3.309
	T-12	-2.107	1.471	1.000	-7.023	2.809
	T-131	-3.104	1.471	1.000	-8.020	1.812
	T-158	3.214	1.471	.971	-1.701	8.130
	T-33	3.467	1.471	.653	-1.449	8.382
	T-97	-2.967	1.531	1.000	-8.083	2.150
T-33	Control	69.248*	2.081	.000	62.296	76.200
	T-11	-5.074*	1.471	.037	-9.990	-.158
	T-12	-5.574*	1.471	.014	-10.490	-.658
	T-131	-6.570*	1.471	.002	-11.486	-1.654
	T-158	-.252	1.471	1.000	-5.168	4.664
	T-32	-3.467	1.471	.653	-8.382	1.449
	T-97	-6.433*	1.531	.004	-11.550	-1.317
T-97	Control	75.681*	2.123	.000	68.586	82.777
	T-11	1.359	1.531	1.000	-3.757	6.476
	T-12	.859	1.531	1.000	-4.257	5.976
	T-131	-.137	1.531	1.000	-5.254	4.980
	T-158	6.181*	1.531	.006	1.064	11.298
	T-32	2.967	1.531	1.000	-2.150	8.083
	T-33	6.433*	1.531	.004	1.317	11.550

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.