ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FACULTY OF SCIENCE

DEPARTEMENT OF BIOLOGY

Evaluation of *Trichoderma* species as Biological control against *Fusarium* wilt (*Fusarium* spp.) on cotton plant (*Gossypium herbaceum*) under *in vitro* condition

A Thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements of the Degree of Master of Science in Biology

By:TsegayAsmekash

Advisor: Tesfaye Alemu (Dr.)

October, 2019

Addis Ababa, Ethiopia

ACKNOWLEDGMENTS

First of all, I would like to express my sincere thanks to the Almighty God for His love, benevolence, forgiveness and generosity.

I am sincerely and heartily grateful to my advisor Dr. Tesfaye Alemu (PhD) for the materials and chemicals support and scientific guidance he showed me throughout my Thesis writing. I am sure it would have not been possible without his unreserved help, moral, love and encouragement.

My genuine appreciation goes to the PhD candidates Ayelign Melesse and Afrasa Mulatufor their constructive comments, guidance, encouragement, and polite behavior. They have devoted much of their time and without their contribution, this paper would not have been in its present complete form.

It gives me great pleasure to present my sincere thanks for the PhD candidates, Mesele Admasie and Alemayehu Dhugasa for their patience and encouragement throughout this study.

My thanks also go to Kedir Beno, Negat Mekonen and Zenebech Ayetenew for their collaboration by providing and supporting materials and information in the mycology laboratory and to all my friends and colleagues.

I would like to thank the farm land manager Ato Hadush Wolu and agronomy expert Ato Gebretsadik Mesfun at Kobabo irrigation and rain fed agricultural area for their technical and professional support during the field work.

I would also like to extend my heartful thanks to Addis Ababa University, Faculty of Science, School of Graduate Studies and Department of Biology for giving me the opportunity to grasp a profound knowledge, and financial support. I am grateful to thank the Ministry of Education for financial support.

Last but not least, I would like to take this opportunity to express my gratitude to my family and friends for their constant supports and encouragements.

Table of content

ACKNOWLEDGMENTS ... 1

Table of content ... ii

List of Tables ... v

List of Figures ... vi

List of Appendices ... vii

Abbreviations ... viii

Abstract ... ix

1. Introduction ... 1

1.1 Statement of the problem ... 2

2. Objectives ... 3

2.1 Main Objective ... 3

2.2. Specific objectives ... 3

3. Literature Review ... 4

3.1 Global Status of Cotton and origin ... 4

3.2 Cotton Production and importance in Ethiopia ... 5

3.3 The biological nature of cotton crop ... 6

3.3.1 Taxonomy, Geographic origin and Distribution ... 6

3.3.2 Climatic Condition and Soil Requirements ... 6

3.4 Reproductive Biology of cotton ... 7

3.4.1 Growth and Development ... 7

3.4.2 Floral Biology ... 8

3.4.3 Pollination and Fertilization ... 9

3.4.4 Pollen Dispersal ... 9

3.4.5 Seed Dispersal ... 10

3.5 Major Diseases of Cotton ... 10

3.5.1 Fusarium Wilt (*Fusarium oxysporumf.sp. vasinfectum*) of Cotton (*Gossypium*) ... 10

3.5.2 History of the Disease ... 11

3.5.3 Wilts Distribution ... 12

3.5.4 The Pathogen (*Fusariumoxysporum*) 13
3.5.5 The evolution of *Fusarium oxysporum* 14
3.5.6 Nomenclature, Taxonomy and Morphology of *Fusarium oxysporum* 14
3.5.7 Environmental Factors 17
3.6 Mechanism of *Fusarium Species* to Cause Disease 18
3.6.1 Development of the Disease 19
3.7 *Fusarium* Pathogenicity and Pathogenicity Factors 20
3.7.2 Differences between *Fusarium* and *Verticillium* wilt symptoms 22
3.8 Economic importance of *Fusarium* 23
3.9 Disease Management of *Fusarium* wilt of cotton 23
3.9.1 Biological control 25
3.9.2 Characteristics of Biological Control Agents 26
3.9.3 Antibiotic-mediated suppression 26
3.9.4 Mechanism of action of *Trichoderma* species as bioagents 27
3.9.5 Formulation and application methods of BCA 28
4. MATERIALS AND METHODS 30
4.1 Descripton of study areas 30
4.2 Sample collections 30
4.3 Experimental site 31
4.4 Isolation of *Fusarium*wilt pathogen from plant tissues 31
4.4.1 Culture re-isolation and purification 31
4.5 Morphological Identification of *Fusarium* isolates 31
4.6 Pathogenicity test 32
4.6.1 Preparation of spore suspension 32
4.6.2 Inoculation to detached leaves and greenhouse seedlings 32
4.6.3 Re-isolation of isolated fungal pathogens 32
4.7 *In Vitro*evaluation of *Trichoderma*against *Fusarium* wilt of cotton 32
4.7.1 Dual Culture Method 32
4.7.2 Growth Inhibition Analysis 33
4.7.3 Volatile Metabolite Inhibitory Bioassay 33
4.7.4 Nonvolatile Antibiotic Inhibitory Assay 33

4.8 Preparation of potato dextrose broth (PDB) media with different pH values ... 34
4.8.1 Sporulation of *Trichoderma* species at different pH values ... 34
4.8.2 Effect of temperature on the isolated *Trichoderma* sp. ... 34
4.9 Statistical analyses ... 34
5. RESULTS ... 35
5.1 Isolation of the Fusarium wilt pathogen from plant tissues ... 35
5.2 Morphological Identification of *Fusarium* isolates ... 35
5.2.1 Cultural pigmentation ... 35
5.2.2 Slide culture and Microscopic features of *Fusarium oxysporum* ... 36
5.3 Pathogenicity test ... 36
5.3.1 Re-isolation of isolated fungal pathogens ... 37
5.4Growth Inhibition Analysis of dual culture antagonistic bioassay ... 37
5.5Growth Inhibition Analysis of volatile and non-volatile metabolites bioassay ... 38
5.6Growth analysis of *Trichoderma* species at different temperature level ... 39
5.7 Sporulation of *Trichoderma sp*. at different pH values ... 40
6. Discussion ... 42
7. Conclusions and Recommendation ... 44
7.1 Conclusions ... 44
7.2 Recommendations ... 44
8. References ... 45
Appendix ... 58

List of Tables

Table 1: The taxonomic classification and nomenclature of cotton crops...................................... 6

Table 2: Evaluation of Effects of Trichoderma isolates on growth of isolated Fusarium species (AAUFcot04) in dual culture bioassay. 38

Table 3: Evaluation of Effects of *Trichoderma* isolates on growth of isolated *Fusarium* species (AAUFcot04) in volatile and nonvolatile metabolite compounds 39

Table 4: OD reading and dry mass of the experimental Trichoderma at a given pH level 41

List of Figures

Figure 1 The growth and development of cotton plant 8

Figure 2 Morphological characteristics of *Fusarium* isolates 16

Figure 3 Developmental life cycle of the pathogen 20

Figure - 4: Pathogenicity factors of fusarium and host defense mechanisms 21

Figure 5: Map of Tigray and the study area (Tsegede District) 30

Figure 6: Six Fusarium isolates isolated from cotton samples 35

Figure 7: Colony color of the identified *Fusarium*isolates on PDA. 36

Figure 8 Microscopic features of *Fusarium* 36

Figure 9: Pathogenicity test on detached leaves. 37

Figure 10: The re isolated culture of *Fusarium* 37

Figure 11: dual culture test plates 38

Figure 12 Antagonistic effect of volatile and non-volatile metabolites against the pathogen. 39

Figure 13 : mean radial growth of *Trichoderma* isolates at different temperature level. 40

Figure 14 comparison on radial growth of particular *Trichoderma* at different temperature. 40

List of Appendices

Appendix 1 Descriptive Statistics (dual culture test) 58
Appendix 2 Multiple Comparisons (dual culture) 59
Appendix 3 Descriptive Statistics (volatile compounds) 61
Appendix 4 Pairwise Comparisons (volatile compounds) 62
Appendix 5 Descriptive Statistics (Non volatile compounds) 63
Appendix 6 Multiple Comparisons (non volatile compounds) 64

Abbreviations

BBPM	biologically based pest management
BCAs	biological control agents
CSA	central statistics agency
EIA	Ethiopian Investment Agency
EIAR	Ethiopian institute of agricultural research
FOV	*Fusarium oxysporumf.sp. vasinfectum.*
ICAC	international cotton advisory committee
IPM	integrated pest management
ISR	induced systemic resistance
PGPR	plant growth promoting rhizobacteria
WARC	Werer agricultural research center

Evaluation of *Trichoderma* species as Biological control against *Fusarium* wilt (*Fusarium* species) on cotton plant (*Gossypium herbaceum*) under *In vitro* condition

TsegayAsmekash*, Tesfaye Alemu[1], Ayelign Melesse[1]

*Department of Zoological Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

[1]Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Abstract

In Ethiopia, cotton is one of the most important cash crops that widely grown in the country. The production trend of cotton has been on the decline over the years, mostly as a result of infectious diseases from fungal pathogens. Thus the overall objective of the study was to evaluate local *Trichoderma* isolates for their pathogenicity potential against *Fusarium* isolates. The ability of the isolates to antagonize *Fusarium* isolates was evaluated in *invitro*dual culture assays and using volatile and non-volatile metabolites produced by the isolates.The effect of temperature and pH on the growth of *Trichoderma*isolates was also evaluated under a pH value ranging from 4.5-7.5 and level of temperature at 4°C, 25°C and 37°C.The inhibition test effects were analyzed using the analysis of variance (ANOVA) and the significance of the means was evaluated by Tukey's HSDtests. Out of27 isolates one isolate wasmorphologicaly identified as *Fusarium* pathogen isolate then selected and subjected to pathogenicity and antagonize test effects.The pathogenicity result showed the typical symptoms of the pathogen on the inoculated detached leaves and seedlings.Among the four*Trichoderma*isolates, T131 showed the highest inhibitory 78.89% effect against the pathogen at based on dual culture assay. Use of volatile and non-volatile metabolites produced by the *Trichoderma* isolates also confirmed that there was a production of inhibitory substances.The*Trichoderma* isolatesT131 (48.14%) and T136 (52.89%) showed the highest inhibitory effect against the pathogen.It is clearly observed fro the results of *Trichoderma*isolates were successfully grown at 25°C and 37°C.Similarly,*Trichoderma*isolates sporulated at different pH values. However, the pH change was not significant except in *Trichoderma* isolate GimT7. In the present study, T 131 were found to be the best isolate against cotton wilt pathogen (*Fusarium spp*).

Keywords: Antagonistic activity, secondary metabolites, dual culture, pathogenicity

1. Introduction

Cotton crop provides the world's premier source for natural fibers, which are mainly used in the manufacture of a large number of textiles. Low-quality fiber can be used for manufacturing felt, mattress filling and special paper, and the processed cellulose is used for various consumer products such as toothpaste, lipstick, ice cream and mayonnaise. There also is a range of applications in the chemical industry (Vreeland, 1999; Wakelyn*et al*., 2007a&b).

The seeds, even though extensively and intensively used worldwide as well, tend to be regarded as a secondary product or byproduct. The seeds are used to obtain edible oil, which is considered to be of very good quality within the range of vegetable oils (O'Brien *et al*., 2005); as chaff for livestock feed; and as high-protein cake and flour, which are used mainly for livestock feed. The flour is sometimes used for human consumption (in low amounts, or after extraction of the gossypol or from gossypol-free varieties). Gossypol has been used as a male contraceptive (Coutinho, 2002). Cottonseed oil is of interest as a lubricant and a biofuel (Karaosmano*et al*., 1999).

Ethiopia is believed to be one of the origins of cotton and cotton cultivation is deep rooted in the history of the country's agriculture. Cotton is one of the major cash crops in Ethiopia and extensively grown in the low lands under large scale irrigation schemes. It is also grown on small –scale farms under rain –fed- agriculture (EIA, 2012). Cotton has grown in many of regions in the country. In each region, there are wide potential areas, forinstance, in Tigray 269130ha, in Amhara 678,710 ha, in SNNPR 600,900 ha, in Oromia 407420 ha, Gambella 316,450, Benshangul 303,170ha, Afar 200,000 ha and Somali 225,000ha. (Ethiopian investment agency, 2012).The average annual export of lint cotton in Ethiopia from 1998/99 to 2004/05 was 6,055 tones, whereas the average revenue obtained from sales of this amount was only 52,457,000 Birr (MoARD, 2005).

Cotton is basically a crop of warmer climates. In Ethiopia, a good cotton yield is obtainable from areas varying in altitude from sea level to about 1000m. A large area of the Country particularly the country's potential cotton growing areas such as Omo-Ghibe, WabiShebele, Awash, Baro-Akobo, Blue Nile, and Tekezze river basins lie within this altitude range.Cotton plants are cultivated in a wide variety of soils, but the crop develops best in deep arable soils with good

drainage, filled with organic matter and with a high moisture-retention capacity. Yet cotton is grown in cracking clays in some countries. Cotton crop also performs well on a variety of lighter soils such as loams. Since cotton is a fairly deep-rooted crop, deep soils of 180cm or more are preferred. The Country's potential cotton growing areas have soils of these types (EIA, 2002).Factors that constrain the production of cotton are shortage of improved seed varieties, shortage of technical inputs, absence of extension service, and limited irrigation practices (RATES, u.d).

Diseases in cotton may affect the quality of the fiber and seed as well as the yield and cost of production of the cotton crop (Bell,1999). The main diseases affecting cotton include: seeding disease, fungi wilt diseases (*Fusarium*wiltor*Verticillium*wilt) and leaf spots. *Fusarium* wilt is a destructive disease of cotton (*Gossypium spp*.) in many countries of the world, including Australia, USA, Egypt, Tanzania, and China (Feng *et al*., 2000).Though the management of Fusarium wilt has proved difficult due to prolonged survival of the pathogen in the soil, many strategies have been investigated in the field in different parts of the world (Jimenez-Díaz *et al*., 2011). A fungicide application is one of the most common methods used to control fungal pathogens. However, repeated use may result in the development of fungicide resistance in the pathogen population and may not also be economically justified in the control of this disease. Moreover, its application on the field has undesirable effects on the environment, agriculture, food quality, human and animal health (Aktar*et al*., 2009).

Biological control of pathogenic organisms is an eco-friendly, risk-free alternative method of agriculture (Benítez *et al*., 2004). Among the biological control agents, *Trichoderma* is a fungal genus of cosmopolitan distribution and high biotechnological value as performing effective biocontrol mechanisms (Hermosa *et al*., 2013). Therefore, the current study has initiated on evaluation of Trichoderma species as a biological control agent (BCAs) against *Fusarium* wilt (*Fusariumspecies*) on cotton plant (*Gossypium*) under in vitro condition.

1.1 Statement of the problem

In Ethiopia, spinning and weaving to make cloth's from cotton is perhaps as old as the history of the country. Though written records are scarce, cotton production is exercised in western TigrayTsegedae district by local farmers under small area and large scales like Hiwot agricultural mechanization at Kobabo irrigation and rain fed agricultural sites. Based on the field observation of the researcher and personal communication with agriculture extension

professionals, cotton wilt disease is the main problem in the area. These days, the cotton wilt disease is assumed to be affecting the yield and quality of the cotton,andas a result there are tremendous losses of income gained from such cash crops in the study areas. Therefore, this studyattemptsto identify the fungal disease of cotton (*Fusarium wilt*) and to test the effect of the biological control against this pathogen in order to minimize the degree of the problem in the district.

2. Objectives

2.1 Main Objective

- The main objective of this study is to evaluate and characterize the potential effects of fungal*Trichoderma* species as a biological control agent against *Fusarium* wilt (*Fusarium species*) on cotton plant (*Gossypium sp.*) under *in vitro* condition.

2.2. Specific objectives

- To isolate, characterize *Fusarium* wilt of cotton(*Fusarium species*) and determine the virulence of the isolated pathogen on the cotton plant under the greenhouse condition.
- To evaluate the biocontrol potential of endophytic *Trichoderma* isolates against*Fusarium*wilt of cotton(*Fusarium species*)under in *vitro* conditions.
- To determine the effect of temperature and pH on the mycelial growth and spore yield of *Trichoderma* isolates.
- To evaluate the action of secondary metabolites on controlling the growth of the pathogen.

3. Literature Review

3.1 Global Status of Cotton and origin

The word cotton refers to four species of the genus *Gossypium L.* apparently domesticated independently in different areas of the world (Sauer, 1993; Brubaker *et al.*, 1999c). The word is derived from the Arabic “quotn”, “kutum” or “gutum” and refers to the crop that produces spinnable fibers on the seed coat (Lee, 1984; Smith, 1995). *Gossypium* (cotton) comprises approximately 50 species worldwide in the arid to semi-arid tropics and subtropics (Fryxell, 1992; Wendel and Cronn, 2003). The main product of the cotton plant is fiber their qualitative characteristics have been valued and analyzed over many centuries.

The global area devoted to cotton production has remained relatively stable over the past three decades, regional changes have occurred. Australia, China, francophone Africa and South Asia have experienced a significant increase in the area under cotton cultivation, whereas cultivated area has shrunk by 40–50% in Brazil and the United States. The advent of new production technologies and better management practices has given rise to an almost 100% increase in average global yields over 30 years, up from 411 kg/ha in 1980/81 to 790 kg/ha of cotton lint in 2013/14 (ICAC, 2014).

Cotton was first cultivated in south Asia and south America. The two species used in south Asia were *G.herbaceum*and*G.arboreum. G.herbaceum* originated in Africa. New world cotton varieties were introduced in to Africa in the 1800s.eventually displacing local varieties (Isaacman, 1996). Cotton can be seen as one important thread of globalization process in Africa. The textile industry was one of the first manufacturing activities to become organized globally, with mechanized production in Europe using cotton from various colonies (Isaacman& Roberts, 1995) In 2007, cotton was grown in 90 countries. In 2006/07, the four main producing countries were China, India the USA and Pakistan and accounted for approximately three quarters of world output. If we added Uzbekistan and Brazil, six countries would account for 83% of world cotton production (EIA, 2012).

3.2 Cotton Production and importance in Ethiopia

In Ethiopia, spinning and weaving to make cloths from cotton is perhaps as old as the history of the country. Though written records are scarce, it is widely believed that Ethiopians wore clothes woven from cotton fibers centuries ago. Still about 85% of the total population living in rural areas of the country, satisfies a significant part of its textile needs from the traditional non-industrial sector. Cloth's that are woven from cotton are popular also in urban areas of the country (MulatDemeke*et al.,* 2004). However, the amount of cotton exported and the amount of revenue generated from the export is low. MulatDemeke*et al*. (2004) indicated that the average yearly domestic production of lint cotton during the period 1996/97-2000/01 was only about 29,849.7 tons. Of this amount, 24,861.0 tons (nearly 83% of the total produced) were destined for the domestic market and only 4,989 tonnes (that is 16.9 %) were exported.

The major cotton growing areas in Ethiopia include the Awash River basin, Arbaminch, Sile, Abaya, Woito, Beles In The North, Metema and humera in the north west. Large potential areas also exist in the western, southern and eastern parts of the country.the total area under cotton production is not exactly known, but the area under the farmer state farms was 42,584 hectars (WARC,2000). The share of small scale cotton procedures was undetermined and thus no survey works were done to determine the share both in production & area coverage. According to the information obtained from USAID Ethiopia (1994), the total area covered by small scale cotton producers in the 1993/94 cropping season was 56000hecatrs.

Ethiopia annually produces approximately 220,000 tons of cotton (central spastic's agency). Most of the cotton production in Ethiopia is from small scale farmers who cultivate about 39,600 hectares annually. The total area under cotton plantation by the private owned enterprises is 54,000 hectares (Ethiopian Investment Agency 2012).Acordingly, the cotton potential areas of Ethiopia are Tigray (269130 hectares),Amhara (678710 hectares),SNNPR (600930 hectares), Oromiya (407420 hectares)Gambella (316450 hectares),Benshangul (303170 hectares) Afar (200000 hectares) and Somali (225000 hectares). The major problems of cotton production in Ethiopia include lack high yielding and widely adaptable varieties, inspect pests and disease management techniques, crop and weed management practices (WARC, 2000).

3.3 The biological nature of cotton crop

3.3.1 Taxonomy, Geographic origin and Distribution

Globally, the Gossypium genus comprises about 50 species (Fryxell, 1992). The taxonomic classification of the cotton crop is described in the table below (Table-1). Gossypium herbaceum (African-West Asian Cotton): Native to sub Saharan Africa and Arabia in semi-desert and savanna where it grows as a perennial shrub. It was probably domesticated in Ethiopia or southern Arabia and its cultivation spread to Persia, Afghanistan, Turkey, North Africa, Spain, Ukraine, Turkestan and china.

Table 1: The taxonomic classification and nomenclature of cotton crops

Common name	**Cotton**
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Malvales*
Family	*Malvaceae*
Genus	*Gossypium*
Species	*Gossypium herbaceum*

Source: (Fryxell, 1992)

The place of origin of the genus *Gossypium* is not known, however the primary centers of diversity are west-central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). DNA sequence data from the existing *Gossypium spp.* suggests that the genus arose about 10-20 million years ago (Wendel & Albert, 1992; Seelanan*et al.*, 1997).

3.3.2 Climatic Condition and Soil Requirements

3.3.2.1 Climate change

The basic conditions required for the successful production of cotton include a long frost-free period, a temperature range of 18–32°C and 600–1200 mm of water over the growing cycle which typically lasts 125–175 days (FAO, 2012). Cotton exhibits a certain degree of tolerance to salt and drought and it is therefore growing in arid and semi-arid regions. However, higher and

consistent yield and fiber quality levels are generally obtained with irrigation or sufficient rainfall.

Cotton is a perennial plant by nature, but has long been grown as an annual crop. Varieties grown commercially today to four species of *Gossypium* which is *G. hirsutum*or upland cotton, produces the bulk of cotton worldwide and G. barbadense comes in second (scauhry and Guitchounts, 2003). Cotton is grown mainly in the longitudinal band between 30°N and 32 ^{O}S. In addition to this cotton needs favorable growing conditions with respect to temperature, sunlight (shine) and soil moisture. A marked dry season is also essential for the balls to open properly and harvesting.

3.3.2.3 Soil requirements

Cotton plants are cultivated in a wide variety of soils, but the crop develops best in deep arable soils with good drainage, filled with organic matter and with a high moisture-retention capacity. Yet cotton is grown in cracking clays in some countries. Cotton is a salt-tolerant plant, with *G. barbadense* more salt tolerant than *G. hirsutum* (Ashour and Abd-El'Hamid, 1970). Salinity stress nonetheless has adverse effects on germination and emergence (Ashraf, 2002); the most common stress effect is the general stunting of the plant's growth (Cothren, 1999). In Ethiopia the typical cotton soils are heavy, dark, often cracking soils. Cotton crop also performs well on a variety of lighter soils such as loams. Since cotton is a fairly deep-rooted crop, deep soils of 180cm or more are preferred. The Country's potential cotton growing areas have soils of these types (EIA, 2002).

3.4 Reproductive Biology of cotton

3.4.1 Growth and Development

The growth of cotton plant starts with germination of seed and it depends on the availability of soil moisture, temperature and oxygen. Germination begins with the entry of moisture into the seed and embryo via the chalazal aperture at the seeds' apex (Christiansen and Moore, 1959). The seed/embryo then begins to swell as it absorbs moisture. Under favorable conditions, the radicle (root tip) emerges within 2-3 days from the seed and newly germinated seedlings emerge above the soil 5-6 days after emergence of the radicle (Oosterhuis and Jernstedt, 1999). The first cotton leaf appears 10-12 days after emergence and leaf development reaches its peak about three weeks after the first buds are formed (Fig-1). The first flower-bud appears on the lower fruiting branch 35-45 days after emergence, depending upon prevailing temperatures. The other

flower buds follow at regular intervals until shortly before flowering ceases. The time taken between the appearance of the first flower bud and opening of the flower may be between 25-30 days. The emergence of a large number of flowers is seen in certain period and thereafter it declines. During the peak period of flowering the vegetative growth is almost negligible and once the rate of flowering declines the vegetative growth restarts. Period of flowering is reduced by late sowing, strong plant competition and moisture stress.

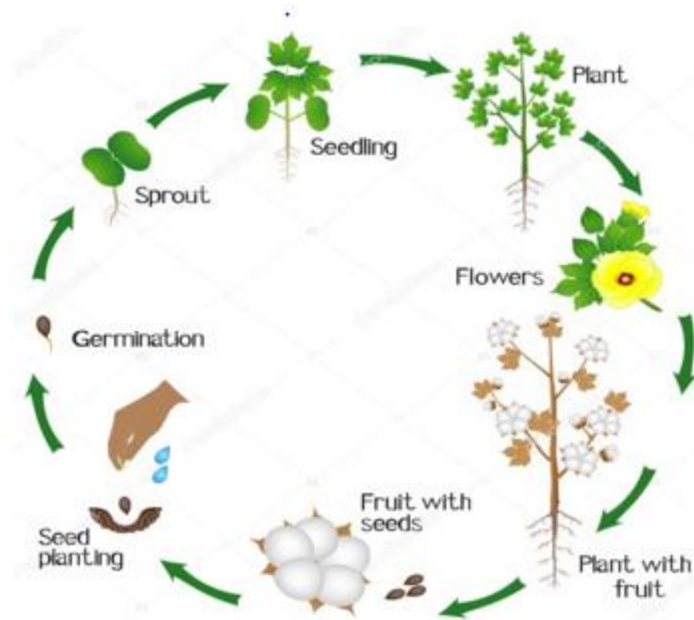

Figure 1 the growth and development of cotton plant

Source: dreamstime.com

3.4.2 Floral Biology

Cotton flowers are extra-axillary, terminal and solitary and are borne on the sympodial branches. The flower is subtended by an involucre of usually three unequal leaves like bracts. Bracteoles, alternating with the bracts on the inside of the involucre or standing on either side of the small bract, may be present. The calyx, consisting of five undiverged sepals, is persistent and shaped as a shallow cup. The calyx adheres tightly to the base of the boll as it develops. The corolla is tubular, consisting of five obcordate petals alternating with calyx lobes and overlapping the next one in the series in a convoluted manner. In some species, a spot of purple, sometimes called 'petal spot', is found on the claw (base) of the petals. On the first day after anthesis, the corolla changes into pinkish blue and then into the red during the succeeding days. It withers and falls off on the third day, together with the staminal column and stigma leaving the ovary, calyx and

involucre intact. The stamens are numerous and united to form a tubular sheath which surrounds the pistils except for the exposed portion of style and stigma at the tip. The pistil consists of 3-5 undiverged carpels corresponding to the locale composition of a fully mature dehisced boll. The ovules are attached to parietal placenta of each locule. The style varies in length and splits near the apex into three, four or five parts depending on the number of carpels.

3.4.3 Pollination and Fertilization

Cotton pollen is relatively large, heavy, sticky and watery and thus wind is not a factor in the pollination of cotton. Cross-pollination in cotton may vary from zero to more than 20 percent. Many insects, especially honey bees are attracted to the cotton flowers and they are active in cross-pollination. Pollination takes place usually in the morning during the opening of a flower and anther dehiscence. Fertilization takes place between 24-30 hours after pollination (Govila, 1969). Corolla along with anthers and filament, drop from the fertilized ovary. Initially the boll development is slow and later the growth rate is rapid and steady. About 40-50 days are required for fertilization to boll bursting, maturation of fibers and seed formation.

3.4.4 Pollen Dispersal

The pollen dispersal depends upon the insect activity and environment in which the parents are grown. The amount of cross pollination depends upon the relative abundance of pollen-carrying insects than any other factor. Generally cross pollination occurs in close vicinity; however, insects may carry the pollen up to several hundred meters. In case of cotton, dispersal studies have consistently demonstrated that when outcrossing occurs, it is localized around the pollen source and decreases significantly with the distance (Thomson, 1966; Galal *et al*., 1972). There are approximately 10,000 pollen grains in a flower. Under normal conditions, the pollen grains are viable upto 24 hours and thereafter lose potency and fail to effect fertilization (Govila and Rao, 1969). Honey bee (*Apis mellifera L.*) is the main vector for pollination in cotton, apart from these honey bees (A. dorsata, A. Florea, A. indicia), bumble bees (*Bombus sp*.), leaf cutting bees (*Hymenoptera megachilidae*) and a few dipterans help in pollination. The main pollinating insects differ due to distribution of insects and ecological conditions and their capability also differ on account of their visiting behavior and body size. Fully pubescent insects such as yellow breast wood bees, heavy flower wasp and black spiny tibial bee are also highly efficient for cross - pollination.

3.4.5 Seed Dispersal

As cotton does not generally reproduce vegetatively (Serdy*et al*., 1995), spread within the environment occurs by seed dispersal. Dispersal of cotton seeds is a physical process. Observations of dispersed seeds and the occurrence of volunteer plants in northern Australian cotton trials indicated that delinted black seed has the lowest risk of unintentional spread within the environment (OGTR, 2002). When dispersal of black seed occurs, it is associated with spillage at sowing in cotton production areas. Fuzzy seed is commonly used as livestock feed and therefore has a high potential for dispersal to noncotton production habitats. Unprocessed 'seed cotton' that retains all of the fibers attached to the seed coat, also has a high potential for dispersal within the environment. Volunteers from dispersed seed cotton are relatively common in irrigated channels and drains and along roadsides. Seed cotton spillage during transport of cotton modules also leads to establishment of roadside volunteers. However, following dispersal, seeds that do not germinate are removed by seed predators or by rotting.

3.5 Major Diseases of Cotton

There are Various types of bacterial, fungal and viral diseases affecting the cotton crop. Such diseases include: i) Root rot (*Rhizoctonia bataticola and R. Solani*), ii) Cotton Bacterial blight (*Xanthomonas axanopodis*), iii) *Alternaria* leaf blight (*Alternaria macrospora*), iv) Grey mildew (*Ramularia areola*), v) Powdery mildew (*Leveillulataurica*), vi) Cotton Leaf curl virus (*Bemisiatabaci*), vii)*Verticillium* wilt (*Verticillium dahliae*), viii) *Fusarium* wilt (*Fusarium oxysporumf.sp. vasinfectum*). Seedling diseases caused by fungi are also the most widespread and devastating biotic stresses that subsequently affect cotton yield (Aly et al., 2000; ElSamawaty, 2004; Nehl et al., 2004). *Fusarium* species are among the most common fungi associated with cotton seedlings damping-off and frequently isolated from diseased seedlings (Palmateer*et al*., 2004; Costa *et al*., 2005; El-Samawaty*et al*., 2008).

3.5.1 Fusarium Wilt (*Fusarium oxysporumf.sp. vasinfectum*) of Cotton (*Gossypium*)

Cotton is a globally important crop used for both its natural fiber and seed. *Fusarium* wilt, caused by the fungus *Fusarium oxysporum f. sp. vasinfectum*, is a major disease of cotton capable of causing significant economic loss. The fungus persists in soil as *chlamydospores* and in association with the roots of susceptible, resistant and non-cotton hosts as well as in seed. Management of *Fusarium* wilt is difficult and most successfully achieved through the use of resistant cultivars and pathogen-free cotton seed. The pathogen (*Fusariumoxysporum*) causing

wilt is soil borne and is present all over the growing area, but only attack whenever suitable conditions (mainly high temperature) prevails (Mustafa, 1983).The wilt comprises an important group of plant diseases in which the plant suffers from dehydration and show symptoms of drought (Bilgram and Dube, 1976). Vascular wilt disease caused by fungi are usually highly destructive whether they occur in cultivated crops or in indigenous species (Mace *et al*., 1981). Wilts occur as a result of the presence and activities of the pathogen in the xylem vessels of the plant (Agrios, 2005).

Symptoms of *Fusarium* wilt can appear at any stage of crop development depending on inoculum density, temperature, and host susceptibility. At a high inoculum density or when infection initiates from the seed, plants may be killed at the seedling stage (Hillocks, 1992). In mildly-affected or older plants, the lower leaves will show symptoms, but the plant will survive, although with reduced vigor. From visibility of the disease, the most diagnostic symptoms of *Fusarium* wilt is the loss of turgor resulting in wilt and a brown discoloration of the vascular tissue. This discoloration is localized in the cortical tissue of the vascular system of the upper tap root and lower stem when cut at a diagonal or in cross-section (Davis*et al.,* 2006)

3.5.2 History of the Disease

Fusarium wilt of cotton is caused by *Fusarium oxysporumf.sp. vasinfectum* (FOV) the main symptoms being wilting, stunting, chlorosis and die back. In the field, development of *Fusarium* wilt is influenced by the virulence of the fungus, susceptibility of the crop, soil type and fertility, weather conditions and interactions with other organisms, including nematodes. *Fusarium* wilt has been reported in East Africa, includingKenya, where it reduces yields and affects fiber quality (ICAC, 2003).

The wilt comprises an important group of plant diseases in which the plant suffers from dehydration and show symptoms of drought (Bilgram and Dube, 1976). Vascular wilt diseases caused by fungi are usually highly destructive whether they occur in cultivated crops or in indigenous species (Mace *et al*., 1981). Wilts occur as a result of the presence and activities of the pathogen in the xylem vessels of the plant (Agrios, 2005).

Fusarium wilt is a destructive disease of cotton (*Gossypium* spp.) in many countries of the world, including Australia, USA, Egypt, Tanzania, and China (Feng *et al*., 2000). The disease is caused by the soil inhabiting fungus *Fusariumoxysporumf. sp.vasinfectum* (G.F. Atk.) Snyder & Hansen

(FOV) (teleomorph: *Neocosmosporavasinfecta*), which causes vascular wilt in susceptible cotton (Watkins, 1981; Chen et al., 1985; Hillocks, 1992; Davis *et al*., 1996) and okra cultivars (Aguiar *et al*., 2013). *Fusarium* wilt of cotton was first observed by Atkinson (1892) in the USA. The first report of this disease outside the USA was done in Egypt, where it was rapidly disseminated after the release of the susceptible cultivar 'Sakal' during the 1920s (Fahmy, 1927).

Currently, up to eight races of FOV are recognized worldwide with most of them being geographically isolated (Abd-Elsalam et al., 2004; Abo *et al*., 2005). The determination of FOV races depends upon the analysis of their virulence profile on a set of differential cotton lines/ species and up to five non-cotton hosts (Davis *et al*., 1996). In Egypt, the occurrence of FOV race 3 has been documented in the Nile Valley, where it remains one of the most damaging pathogens on *G. barbadense* cultivars (Watkins, 1981; Abd-Elsalam*et al*., 2004). To date, race 3 is the only one found in Egypt. This race was also reported attacking *G. barbadense* in the former Soviet Union (Watkins, 1981).

3.5.3 Wilts Distribution

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* (Plant Health Research and Diagnostics, 2007) which 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, prennial, ornamentals, and fruit and forest tree (Agrios, 1988). 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) prevails (Mustafa, 1983). There are four genera of fungi that cause vascular wilts: *Ceratocystis, Ophiostoma, Fusarium and Verticilum*. Each of them causes disease on several important crops, forest and ornamental plants.

In Ethiopia Cotton wilt diseases are caused by two soil-borne vascular pathogens, *Verticillium dahliae and Fusarium oxysporum*. The diseases are known to occur occasionally in Tendaho, less frequently in Arbaminch and sporadically in the Middle Awash cotton farms. *Verticillium* wilt is recorded only from Sile farm (Semen Omo Agricultural Development Enterprise), while *Fusarium* wilt was common in most farms of the Middle Awash and rain-fed cotton (GeremewTerefe, 1990).

3.5.4 The Pathogen (*Fusariumoxysporum*)

Fusarium is among the most diverse genera and very important pathological (Synder and Hansen, 1989). *Fusarium oxysporum*is an asexual fungal species that include human and animal pathogens and a diverse range of non-pathogens. Pathogenic and nonpathogenic strains of this species can be distinguished from each other with pathogenicity tests. The fungus has septation and profusely branched growth on potato dextrose agar (PDA) at 25 ºC (Booth, 1971).

Initially, it produces white mycelial growth turning light buff or deep brown later and fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, whitish/cream, dark purple, light orange) may be observed in culture on solid medium. The color of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink or purple and on the reverse, it may be colorless, tan, red, dark purple, or brown (Kontoyiannis*et al*., 2000).

The *Fusarium* genus comprises a wide variety of filamentous fungi recognized as plant, animal and human pathogens and soil saprophytes (Leslie and Summerell, 2006). Some fusaria cause animal and human diseases directly, e.g. keratitis or indirectly by producing mycotoxins such as trichothecenes and fumonisins (Moretti, 2009). Fusaria are widely distributed around the world and can survive for a long time in all types of soils as they are able to utilize a wide range of organic matter (Nelson *et al*., 1981).

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 rots (Leslie &Summerell, 2006). The fungus can be found worldwide and is considered as the most widely dispersed and most economically important *Fusarium* species (Leslie &Summerell, 2006). Over 70 formea speciales and races have been described in*F.oxysporum*, although only a few can be differentiated using molecular techniques (Leslie &Summerell, 2006, Booth, 1971).

3.5.5 The evolution of *Fusarium oxysporum*

Fungi, like all living organisms, have the ability to adapt in response to changing or new environments. Environmental changes exert selective pressure on an organism (McDonald, 1997), and only individuals that adjust to change are able to succeed. The capacity of populations of pathogens to adapt is determined, in part, by their diversity (McDonald and McDermott, 1993). As the gene pool that a population can sample increases, so too does its adaptability to a changing environment or a new host genotype. It is generally assumed that the dynamics of the evolution of fungi are determined by five evolutionary forces: mutation, natural selection, genetic drift, gene flow and mating system (McDonald and Linde, 2002). Mutations are changes that take place in DNA base sequences, which are thought to be rare and change the genetic constitution of a population slowly. Natural selection typically favors genotypes with a reproductive advantage, while genetic drift refers to changes in gene frequency within a population that take place due to chance alone. Gene flow represents the movement of gametes, individuals or populations, from one area to another, potentially expanding the range of novel pathotypes.

The final evolutionary force that brings about change in *F.oxysporum* and its populations is reproductive strategy. Since no teleomorph is known for this fungus, meiotic recombination is not believed to be involved in generating new genetic combinations. Rather, asexual organisms like *F. oxysporum* are thought to evolve by means of mutations only and potentially through the processes of Para sexual recombination (i.e. A non-sexual mechanism for creating new genetic combinations) (Kuhn*et al*., 1995).*Fusariumoxysporum f. sp. vasinfectum*is a ubiquitous, asexual soil borne fungus, which may live saprophytically or as a destructive pathogen of many crops including cotton. The fungus attacks the cotton plants, mostly at seedling stage. The fungus invades the plant through root wounds and then infects the vascular system, resulting in wilt symptoms (Hillocks & Bridge, 1992). The disease is highly destructive and causes great losses in cotton as it affects both yield and fiber quality. Disease incidence was reported in more than five percent of sampled fields (Hillocks &Kibani, 2002).

3.5.6 Nomenclature, Taxonomy and Morphology of *Fusarium oxysporum*

Agrios (2005) reported that the genus *Fusarium* belongs to the class Fungi Imperfectii (Deuteromycetes or mitosporic fungi). It comprises of many species and many forms within species. *Fusarium* species were traditionally classified in the Deuteromycotina/Fungi Imperfectii although affinities to *Ascomycotina* have been established (Table-2). Traditional classification

and identification schemes for *Fusarium* are exclusively based on a morphological species concept derived from cultural characteristics of single-spore isolates grown on special media, shared morphological trait of the anamorph, host range and, to a lesser extent, teleomorph micromorphology (Booth, 1971).

The *Fusarium* genus was first described by Link in 1809. Since then the taxonomy of the *Fusarium* genus has changed considerably. Today it is generally accepted that the genus *Fusarium* is classified under the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order (Leslie &Summerell, 2006). The anamorph is found in the *Deuteromycota,* in the class Hyphomycetes (Leslie &Summerell, 2006). The teleomorphs of the *Fusarium* genus are typically classified in the Gibberella genus, however, there are also a few species with teleomorphs in the genera Nectria, Cosmospora, Hemanectria, Albonectria, Monographella and Plectosporium (Moretti, 2009).

Historically, the differentiation of *Fusarium* species was based solely on morphological characters, and an individual assigned as a type for that species represented the variation inherent in the entire species (Leslie &Summerell, 2006). The morphological characters considered important for identification included the shape and size of macroconidia and microconidia, mode of formation of microconidia, production of *chlamydospores*, and growth rates on selective media (Leslie &Summerell, 2006).

Approximately 1000 species had been described before Wollenweber and Reinking (1935) thoroughly reclassified the genus into 16 sections and 65 species. Since 1935, various attempts have been made to improve the Wollenweber and Reinking classification system. Snyder and Hansen (1940) compressed the 16 sections into nine species, and the species in section Elegans into a single species, *F. oxysporum*. Snyder and Hansen's classification system was eventually replaced by a system proposed by Nelson *et al*. (1983), which attempted to bridge the previous systems. The only species that remained unchanged from Snyder and Hansen's (1940) treatment were *F. oxysporum*and *Fusarium solani* (Martius) Appel &Wollenweber emend. Snyder and Hansen, although recent work has proposed that both taxa represent species complexes (O'Donnell *et al*., 2009; O'Donnell *et al*., 2008). Leslie and Summerell (2006) summarized information for 70 species of *Fusarium*, and this monographic publication was the first for the genus to integrate morphological and phylogenetic information.

3.5.6.1 Morphological Taxonomy

The morphological taxonomy of species in the genus *Fusarium* is based primarily on the structure and abundance of asexual reproductive structures (*chlamydospores*, fields, microconidia and macroconidia) and on cultural characteristics (colony texture, color and cultural aroma) (Booth, 1971; Edel et al., 2000; Nelson, 1991; Nelson *et al*., 1983). *F. oxysporum* is characterized mainly by non-septate microconidia formed in false heads on short monophialides, 3- septate macroconidia formed from monophialides on branched conidiophoresinsporodochia, and chlamydosporeswith a smooth or rough wall appearance formed singly or in pairs (Fig-2). However, identification of this and other species of *Fusarium* can be challenging, because of variation between isolates (Snyder and Hansen, 1940), which reflects both genetic and environmental effects on the phenotypic expression of morphological characters (Nelson, 1991). In addition to lacking stability, morphological characters may not provide the resolution needed to properly circumscribe newly identified species.

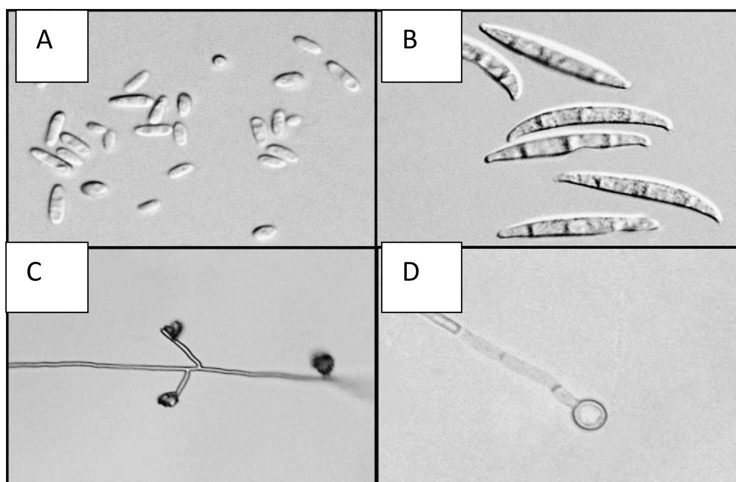

Figure 2 Morphological characteristics of *Fusarium* isolates (A) Oval to kidney-shaped microconidia; (B) sickle-shaped, thin-walled and delicate macroconidia; (C) microconidia produced in false heads on short monophialides; and (D) a single, terminal *chlamydospore*.Source:journal of molecular epidemiology and evolutionary genetics in infectious diseases: PubMed.

Discrimination of *Fusarium* species was traditionally based on morphological characters (morphological species recognition) that enabled the identification of some of the most important pathogens (Moretti, 2009). However, the genus lacks reliable morphological characters (Geiser *et al.*, 2004), and it is therefore very difficult for researchers with little experience to characterize *Fusarium* species based purely on morphological traits. For example, differences in the shape

and size of the macroconidia and microconidia can be subjective and depend upon the environment in which they are produced (Leslie and Summerell, 2006).

3.5.6.2 Biological species recognition

Fusarium species can be recognized based on their ability to, actually or potentially interbreed with other members of the population (biological species recognition), rather than their morphological appearance (Mayr, 1940). For instance, the *Gibberellafujikuroi*species complex was divided into at least eleven different biological species or mating populations (Leslie, 1991). On the other hand, it is difficult to apply the concept of biological species to fusaria because some lacks sexual reproduction. Although several *Fusarium* species, i.e. *F. solani*and *F. Graminearum*, mate in either a homothallic or heterothallic manner, important pathogenic species, including *F. avenaceum*, *F. culmorum*and *F. equiseti*, have no known sexual stage. Phylogenetic analyses (phylogenetic species recognition) can be useful to resolve a species level identification of fungi without a known sexual stage.

3.5.6.3 Macroscopic and microscopic features

The genetic structure of *Fusarium* species is variable and the morphology of the species is influenced by environmental factors. For many of the species, specific conditions are required for optimal morphology manifestations and the tendency to mutate causes difficulties in identification. Most *Fusarium* species grow rapidly on Sabouraud dextrose agar 25°C and produce woolly to cottony, flat, spreading colonies. Sclerotia are masses of hype that remain dormant under unfavorable growth conditions; they may be observed microscopically in some species and are usually dark blue in color (Arora, 1986). In addition, some species have a sporodochium, which is a cushion-like mat of hyphae bearing conidiophores over its surface. This is usually absent in culture. When present, it may be observed in cream to tan or orange color, except for *Fusarium solani,* which gives rise to blue-green or blue sporodochia (Boonk*et al*., 1998).

3.5.7 Environmental Factors

Fusarium wilt is known for being a warm temperature disease. Optimum temperature for spore germination and growth through soil is 25°C (Nelson *et al*., 1981; Nelson, *et al*., 1990), with *F. oxysporum* being able to grow from 10 to 32°C, with severe inhibition above or below these temperatures (Nelson *et al*., 1981; Larkin, andFravel, 2002). Cotton plants may recover from the disease and re-grow if the temperature falls below the optimum for disease development after initial infection.

Individual species of *Fusarium* are limited to specific soil, climate, and biotic conditions. Typically, *Fusarium* wilt of cotton is most damaging in acid, sandy soils (Bell*et al*., 2003) with a pH of 5.0-6.5, and generally less of a problem in heavier clay soils (Larkin, *et al*., 1993). Natural suppression of *Fusarium* wilt disease has been known to occur in many soils (Larkin *et al.*, 1993). This suppression is generally biological in nature, but is also commonly associated with certain physical and/or chemical characteristics such as relatively high pH, clay, and organic matter content (Larkin, and Fravel, 2002). Smith and Snyder (1972) stated that red lateritic clay soils are usually suppressive to *Fusarium* wilt. Though, once a field is infested with FOV the fungus usually sustains itself continuously within the field (Smith, and Snyder, 1975).

3.6 Mechanism of *Fusarium Species* to Cause Disease

Fusarium species are soil borne pathogens, which attack the water conducting vessels of host plants. In soil, fungal colonization of plant roots has been traditionally studied by indirect methods such as microbial isolates that do not enable direct observation of infection sites or of interactions between fungal pathogens and their antagonists (Olivain*et al*., 2006).

The exudates serve as a rich source of carbon (sugar), nitrogen (amino acids) and organic acids, which are generally known to stimulate spore germination (Nelson, 1991; Huisman, 1982). However, what triggers the production of exudates and the actual role of exudates in the germination of spore are not yet understood. *Fusarium* species enter the parasitic phase when any of the propagules or germ tube of spore, penetrates the host through cracks formed by emerging lateral roots, wounds or at the root cap, root hairs or branch roots (Inoue *et al*., 2002).

The penetration process is likely enhanced by certain hydrolyzing enzymes secreted by *Fusarium* (Walter *et al*., 2009). Inside the xylem vessels, the mycelia produce microconidia, which are released to travel upward in the transpiration stream, until trapped in pit cavities or at vessel end walls (Fig-3). They germinate into new hypae and penetrate adjacent vessel elements to continue colonization and increase infection (Schnathorst, 1981). At this stage, *Fusarium* hyphae spread within the cell apoplast, which leads to significant cytological alterations resulting in symptom expression (Walter *et al*., 2009).

A combination of vessel clogging by mycelia, spores (from the fungus) and gels, gums, tyloses and crushing of the vessels by proliferating adjacent parenchyma cells from the host plant in an attempt to defend itself plug vessels and is responsible for the breakdown of the water economy of infected plant (Agrios, 2005). This gives rise to wilting of lower branches, followed by the

entire plant, which eventually leads to death. The new spores can either be returned to the soil when the plant decomposes or disseminated to new plants or areas by wind or water. In the process, conidia are also formed in sporodochia on dead leaves, and hyphae and chlamydospores are also produced extensively. The chlamydospores are returned to the soil, when the diseased plant residues decay. They can remain viable in the soil in their dormant state for several years, and grow upon germination by parasitic or saprophytic colonization of a new host. Certain weeds are symptomless carriers of *Fusarium* (Fassihiani, 2000).

3.6.1 Development of the Disease

The pathogen is a soil inhabitant. Between crops it survives in infected plant debris in the soil as mycelium, spore forms, and as chlomydospores. It spreads over short distances by means of water and contaminated farm equipments, and over long distance primarily in infected transplants or in the soil carried with them. Usually, once an area becomes infected with *Fusarium*, it remains so indefinitely (Agrios, 1997).

When the leaves transpire more water than the roots and stem can transport to them, the stomata close and the leaves wilt and finally die which is followed by the death of the rest of the plant. The spores may be disseminated to new plants or areas by wind, water and other agents. Sometimes, when the soil moisture is high and the temperature relatively low, infected plants may produce good yields. However, in such cases the fungus may reach the fruit of the plants and penetrate or contaminate the seed. Usually, infected fruits decay and drop. If harvested, infected seeds are so light so that they are eliminated in the procedures of extraction and cleaning of the seed and therefore play little roll in the spread of the fungus (Agrios, 1997).

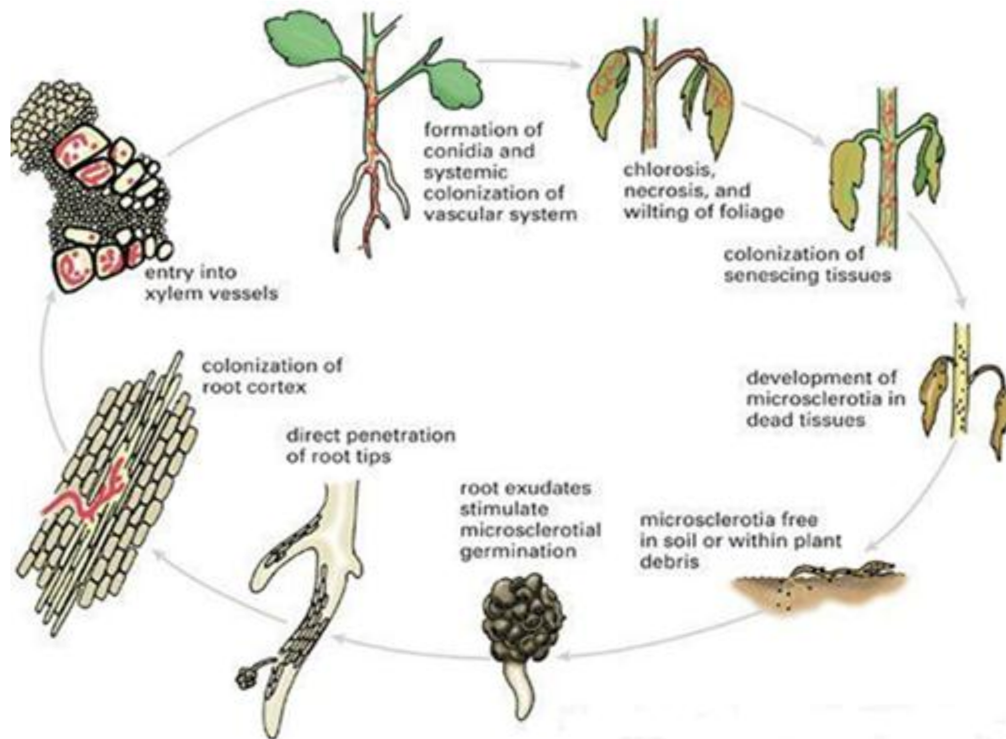

Figure 3 Developmental life cycle of the pathogen

Source: drawing by Vickie Brewster, colored by Jesse Ewing

3.7 *Fusarium* Pathogenicity and Pathogenicity Factors

Pathogenesis is the complete process describing disease development in the host, from initial infection to production of symptoms (Lucas, 1998), and pathogenicity is the ability for pathogenesis. *F. oxysporum* initially penetrates roots asymptomatically; subsequently, it colonizes vascular tissue and triggers massive wilting, necrosis and chlorosis of aerial produce. Certain species-producing toxin, fusaric acid initially infects floral tissue during anthesis, spreads to flower through the central axis of inflorescence, eventually damaging and contaminating grains with toxins (Gardiner *et al*. 2013).

Fusarium pathogens use both general and specific pathogenicity factors/mechanisms to invade their hosts (Fig-4). Hydrolytic enzymes involved in plant cell wall degradation and components of cellular signalling pathways, which are often required for systemic pathogen invasion, comprise general pathogenicity factors, whereas production and secretion of effectors and host-specific toxin are specific pathogenicity factors. The counter defense mechanism of plants plays significant role in pathogenesis and categorized as general and specific (Poppenberger*et al*., 2003). General defense mechanisms encompass production of antifungal proteins and activation

of defense signalling pathways, whereas pathogen-specific include recognition of specific pathogen effectors by plant resistance gene products and detoxification of pathogen-specific toxins (Proctor *et al*., 2007).The specific properties that discriminate endophytic strains from closely related non-endophytic strains have been identified in several studies (Taghavi*et al*. 2010; Mitter*et al*. 2013; Amadou*et al.* 2008; Tisserant*et al*. 2013; Tian *et al*. 2012; Karpinets*et al*. 2014).

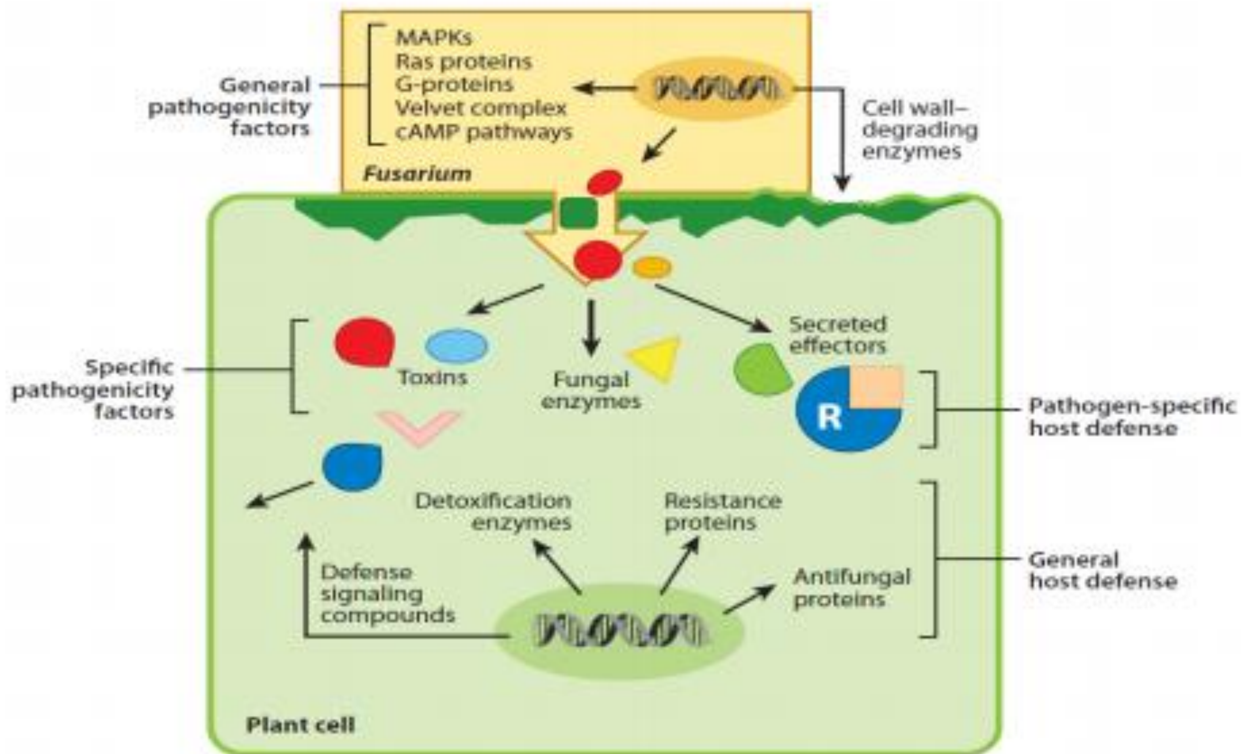

Figure -4: Pathogenicity factors and host defense mechanisms during *Fusarium* plant-interactions (reproduced from Ma *et al*., 2013).

3.7.1 Symptomology and epidemiology of *Fusarium*wilts

The general symptoms of wilts caused by *Fusarium* are stunted growth, yellowing and wilting of the leaves, reddish discoloration of the xylem vessels, visible inside the stem as lines (if the stem is cut open lengthwise) or dots (if it is cut across). Others are white, pink or orange fungal growth on the outside of affected stems, particularly in wet conditions, root or stem decay (Mueller and Beckman, 1988; ICAC, 2003; Miller *et al*., 2011).

The amount and severity of leaf damage that are seen in the field depend on a number of factors, including the distribution of inoculum of the fungal organism in the field, the degree to which the vascular tissue in stems has been damaged by the fungus as it grows within plant tissues, the duration of the fungal "attack" and ability of the plant to re-grow or retain leaves. At the seedling stage or in young plants, cotyledons and leaves wilt and drop, leading to bare stems. Early detection of *Fusarium* wilt can be difficult because early symptoms may resemble some types of seedling disease and symptoms developing much later may be similar to those of other diseases. For example, symptoms are easily confused with those of crown or root rot, stem cankers, pest injury, drought, nutrient deficiency, bacterial and *Verticillium*wilts (Plant Health Research and Diagnostics, 2007).

Past research has shown that impacts of *Fusarium* can also be worse when infections are combined with some types of plant stress that compromise plant health and growth, such as high temperature stress or injury; nematode damage; root injury associated with fertilizer "burn"; anaerobic conditions associated with over-irrigation; moderate to severe water deficits (Morrell and Bloom, 1981; Plant Health Research and Diagnostics, 2007).

3.7.2 Differences between *Fusarium* and *Verticillium* wilt symptoms

Vascular discoloration (brown staining of stem tissue) caused by *F. oxysporum* tends to be darker and more continuous, whereas *Verticillium* causes more of a "flecking"or "spotty" type of stain. The vascular discoloration tends to be seen lower in the plant stem in *Fusarium* than what is seen with *Verticillium* (more in the lower stem, below the cotyledonary node and upper tap root in *Fusarium*). Another interesting difference is that *Fusarium* wilt is favored by warm temperatures while *Verticillium* wilt is favored by cool temperatures. This means that *Verticillium* is typically more damaging following cool weather (organism is more active in growth under cooler conditions), while the *Fusarium* organism is more active in warmer weather. On many, but not all incidences, significant plant foliar and vascular tissue damage occur at earlier growth stages in *Fusarium* than with *Verticillium*, resulting in more stunting of plants with *Fusarium*. Yellow, necrotic areas is more likely to occur at the outer edges of leaves with *Fusarium*, while discoloration can spread across much of the leaf in *Verticillium* (Hutmacher*et al*., 2003)

3.8 Economic importance of *Fusarium*

As well as being common plant pathogens, *Fusarium* species are causative agents of superficial and systemic infections in humans (Mayayo*et al*., 1999). There are species which are highly mycotoxigenic, producing a range of toxins affecting wildlife, livestock and humans (Marasas*et al*., 1984). The genus has a wide distribution and some of its species occur in all major geographic regions of the world (Burgess, 1994). Dietary exposure to *fusarial* toxins causes irreversible tissue damage through biochemical mechanisms that produce pro-oxidative, pro-inflammatory, carcinogenic and/or immune suppressive effects at a cellular level (Baumrucker and Prieschl, 2002). Some toxigenic species have furthermore been implicated as causative agents of life-threatening opportunistic infections in immune-suppressed humans. Mortalities ranged between 50–80% of these cases (Nelson *et al*., 1994).

Several species produce airborne conidia and are common colonizers of stems, leaves and floral parts of plants (Burgess, 1994). Consequently farming practices, such as conservation tillage are likely to increase the level of inoculum of *Fusarium* species (Summerbell *et al*., 1989). *Fusarium oxysporum* is one of the most variable species within the genus. It includes populations that cause vascular wilt diseases (Burgess, 1994) and populations that cause root, crown, tuber, corn, and bulb rots (Nelson *et al*., 1981). The serious wilts, such as tobacco wilts and Panama disease of bananas caused by *F. oxysporum*, are among the most devastating plant diseases in the world.

Fusarium head blight (scab) has recently re-emerged as a devastating disease of wheat and barley throughout the world (Windels, 2000). Undesirable effects of the disease include reduction of grain yield and quality, mycotoxin poisoning in livestock fed with contaminated cereals and mycotoxin carry-over to food products. Species such as *F. moniliforme, F. graminearum, F. avenaceum and F.culmorum* are serious pathogens of *Gramineae* causing pokkah-boeng of sugarcane, bakanae disease of rice as well as, pre- and post-emergence blight of cereals. In addition, *F. graminearum* is a major fungal pathogen of cultivated cereals responsible for billions of dollars in agriculture losses.

3.9 Disease Management of *Fusarium* wilt of cotton

Once established in the soil, the fungus is almost impossible to eradicate (Hillocks and Kiban, 2002). The chlamydospores remain viable in the soil for several years and the pathogen is able to multiply on the roots of many weed and crop species (Smithand Snyder, 1975). Therefore,

management of *Fusarium* wilt in cotton is difficult. An integrated pest management approach specifically for the control of *Fusarium* wilt of cotton has not been described. Some advances have been made in the managing of *Fusarium* wilt with host resistance (Davis, *et al*., 2006); however, there are no immune cultivars.

Cultural methods: Crop rotation is often recommended as a management strategy (Smith, *et al*., 2001). However, rotation to any crop other than cotton prevents an increase in the soil population of *Fusarium*, but may not reduce the number of spores in the soil. *Graminaceous* species have been considered non-susceptible to *Fusarium* wilt, and yet several of them are able to sustain high populations of the fungus on their roots (Hillocks, 1992). Successful chemical management strategies have not been proven. *Fusarium* soil populations have been shown to be decreased by soil fumigation, using a mixture of chloropicrin and methyl bromide (Jorgenson, *et al*., 1978). However, this type of treatment is usually confined to high-value crops grown in relatively small areas.

Physical methods: Increases in soil temperature, which occur under polyethylene sheeting, have been shown to decrease the population of *Fusarium sp*. (Katan *et al*., 1983). Soil solarization under clear plastic for a minimum of 5-6 weeks decreased the incidence of wilt and increased cotton yields in Israel. The beneficial effect was apparent over three seasons (Hillocks, 1992). Ben-Yephet*et al*., (1987) also observed soil solarization reducing the survival of the pathogen in the soil and reducing the incidence of wilt. Although solarization appears to be effective, it is not economically feasible for large-scale cotton production. In addition to the costs associated with implementing solarization, treated fields are out of production for 1 year during the solarization.

Other containment options include tillage, restricting traffic in affected patches, especially when the soil is wet, destroying affected plants and surrounding non-symptomatic plants, and stopping irrigation of affected patches in order to prevent movement of infested soil. The following is not an effective strategy unless weeds are controlled, since many weeds are also hosts of the root-knot nematode (Davidson and Townshend, 1967). Tillage practices impact both fungal and nematode pathogens in the soil (Minton, 1986). Reduced tillage procedures may reduce the spread of the pathogens within a field (Minton, 1986). Because of the ability of the fungus to survive in the soil for extended periods of time, tillage is expected to have little impact.

Management of *Fusarium* wilt must be undertaken with the knowledge of the intrinsic biological features of FOV, which may be classified in four major categories: (i) persistence as long-lived

chlamydospores in soil in association with organic materials or plant residues; (ii) ability to invade and colonize a broad range of host crop plants and weeds; (iii) interactions with nematodes; and (iv) transmission through seeds. Effective management of *Fusarium* wilt should target these biological features in order to reduce the inoculum potential of FOV in soil.

Chemical methods: Soil treatment with broad-spectrum fumigants such as methyl bromide, chloropicrin, or methyl isothiocyanate both alone or in mixtures successfully controlled Fusarium wilt of tomato and increased crop yield (Beckman, 1987). However, the efficiency of soil fumigation is curtailed by either survival of pathogens in soil layers below the depth of effective fumigation, or reintroduction of them through infected planting material or by conidia carried in the air or irrigation water (Katan, 1997). Also, methyl isothiocyanate is prone to enhanced biodegradation in soil by adaptation of microbial populations to use the compound as an energy source.

3.9.1 Biological control

Biological control is an important area of focus in the discipline of Plant Pathology. Every major university with department of Plant Pathology has one or more faculty members conducting basic and/or applied research on biological control organisms (Gardner and Fravel 2002). Biological control agents (BCAs) have been found among the most abundant plant associated microbial genera such as PGPR – Plant Growth Promoting *Rhizobacteria* (*Bacillus*, *Burkholderia*, *Pseudomonas*, *Streptomyces*) and the fungal genera *Trichoderma*. While synthetic toxins have their place in disease control, there is growing awareness that Biologically Based Pest Management (BBPM) fitting in the existing IPM strategies provide more environmentally friendly and economically viable alternatives for agriculture. Whether acting by competitive exclusion, biochemical antagonism or induction of host defenses, BCAs must be well adapted for survival and functional activity in the phytosphere (Gardner and Fravel, 2002).

Biological control is the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control diseases, insect pests and weed populations. The organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA) Kamal and Brian (2006). Biological Control is the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by the mass introduction of one or more antagonists (Baker and Cook, 1974).

More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen (Kamal and Brian, 2006). 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 (Kamal and Brian, 2006).

3.9.2 Characteristics of Biological Control Agents

The BCAs exhibit different modes of action and hence, a good testing program should elucidate all the mechanisms involved in the biocontrol activity of the BCA. Apart from biocontrol ability, the BCAs possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Harman *et al*., 2004; Kok and Victoria, 1999). These traits are useful for good BCA as they help in the establishment of the BCA in a given agro-ecological region.

3.9.3 Antibiotic-mediated suppression

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. Most microbes produce and secrete one or more compounds with antibiotic activity. Antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens. Several biocontrol strains are known to produce multiple antibiotics, which can suppress one or more pathogens. Bacillus cereus strain UW85 is known to produce both zwttermicin and kanosamine. The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens (Pal and Mcspadden Gardener, 2006).

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) *Trichodermaspp*. 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).

Mycoparasitic activity of *Trichoderma spp*. against phytopathogenic fungi and oomycetes due to lytic activity of cell wall-degrading enzymes has been widely studied. In addition to mycoparasitism, other mechanisms have been proposed to account for biocontrol of plant disease by *Trichoderma spp*. including the induction of resistance in the host plant and competition for nutrients and potential infection sites (Harman *et al*., 2004).

*Trichoderma*is widely used in agriculture, and some of the most useful strains demonstrates a property known as rhizosphere competence, the ability to colonize and grow in association with plant roots (Harman, 2000). *Trichoderma harzianum*and *T. viride* are the most studied of all the *Trichoderma* species for biological control and the most effective in reducing diseases caused by soil borne plant pathogens (Baker, 1987, Tesfaye Alemu and Kapoor, 2004).

3.9.4 Mechanism of action of *Trichoderma* species as bioagents

The benefits of using *Trichoderma* in agriculture are multiple, and depending upon the strain the advantages for the associated plant can include: (i) colonization of the rhizosphere by the BCA (‘‘rhizosphere competence’’), allowing rapid establishment within the rhizosphere of a stable microbial community; (ii) control of phytopathogenic and competitive micro flora or fauna by using a variety of mechanisms; (iii) overall improvement of the plant health; (iv) plant growth promotion, by stimulation of above and below ground parts; (v) enhanced nutrient availability and uptake, and (vi) induced systemic resistance (ISR) similar to that stimulated by beneficial rhizobacteria (Harman *et al*., 2004).

Trichoderma biocontrol strains utilize numerous mechanisms for both attacking other soil organisms and enhancing plant and root growth (Harman, 2000; Harman *et al*., 2004; Vinale*et al*., 2008). The colonization of the root system by rhizosphere competent strains of Trichoderma results in increased development of root and/or aerial systems and crop yields (Harman and Kubicek, 1998; Yedidia*et al*., 2001). *Trichoderma* has also been described as being involved in

other biological activities such as the induction of plant systemic resistance and antagonistic effects on plant pathogenic nematodes (Sharon *et al*., 2001).

Safety-many biocontrol agents are closely related to opportunistic human pathogens. Examples of biocontrol organisms of questionable safety abound. *Pseudomonas aeruginosa*, a biocontrol agent of gray leaf spot on turf is a virulent opportunistic pathogen infecting surgical wounds and severe burns. *Burkholderiacepacia*, a highly successful biocontrol agent of pea root rot and other diseases, is associated with opportunistic lung infections of patients with cystic fibrosis. *Trichoderma viride* is an opportunistic human pathogen and is on the biological warfare list in some countries Handelsman (1996). Therefore, it is important to carry on safety test before application of biological control agents.

3.9.5 Formulation and application methods of 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 proposals 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. minitansproduct (De Vrije *et al.*, 2001).

Unless inocula of BCAs are used immediately following production, cells or biomass are usually dried and formulated as products capable of storage, distribution and application (Fravel, 2005). Drying can be done by a range of different methods, including air- and freeze-drying, drying on silica gel and spray- and fluid bed-drying. These methods reduce the metabolic rate of the inoculum by removing the available water, which tends to preserve the inoculum with high viability depending on the BCA. Once the inoculum is dried, it is usually mixed with various components such as carriers, bulking agents, diluents and food bases. BCAs have been formulated as dusts, gels, emulsions, prills, pellets and granules for seed treatments, dips, wettable powders and sprays for application to aerial plant parts, and drenches for incorporation

into soil and growing media (Fravel*et al.*, 1998). Most of the works on formulation closely involve agrochemical, biotechnology or seed-treatment companies and, unfortunately, tends not to be published. The final formulated product should be convenient to use, safe to handle and have an adequate shelf life with stability for at least 1 year. Other desirable characteristics of a formulation include compatibility with application machinery, and ease of integration into integrated pest and disease control systems (Fravel*et al.*, 1998).

4. MATERIALS AND METHODS

4.1 Description of study areas

The study was conducted in Tsegedie district Kobabo irrigation and rain-fed agricultural areas which is located in western zone, Tigray region between 13°14′21″ and 13°44′46″ north latitude and 36°27′44″ and 37°45′5″ east longitude within the tropics.In these areas, wide range of crops most commonly cotton are growing under three selected sites, namely site 1, site 2, and site 3 each of them are far apart at a distance of about 4.5-5km.The altitudinal variation ranges from 500 meters above sea level (masl) at Dansha to 3000 masl at Cheguarcudo. Annual rainfall of the District ranges from 600 mm to 1800 mm while the annual temperature ranges from 27°C to 42°C in the lowland areas (Kolla) and 10°C to 22°C in both midland and highland areas of the study site.

Figure 5: Map of Tigray and the study area (Tsegede District)

Source: *www.feg-consulting.com/---/Tsegede.pdf;www.gov.et/pdf accessed on 27/05/2015*

4.2 Sample collections

Samples of diseased local varieties of cotton plant parts (roots, stems, leaves) were collected from the study area (Tsegede District). Root samples were kept in plastic sample bags, whereas

leaf and stem samples were handled in the envelopes sample bags. The types of samples and sites from where the samples were collected and designated by KCL(Kobabo cotton leaves 1,2,3), KCS(Kobabo cotton stem 1,2,3), KCR(Kobabo cotton root 1,2,3)and numbers indicate the sites.

4.3 Experimental site

The collected samples were kept in plastic sample bags and labeled the type of sample, the site from where it was collected and transported to the mycology laboratory, Department of Microbial Cellular and Molecular Biology, AAU for isolation and characterization of the fungal pathogen iaolatesof cotton causing wilting (*Fusarium*spp).

4.4 Isolation of *Fusarium* wilt pathogen from plant tissues

Diseased plant parts of cotton (roots, leaves and stems) were thoroughly washed with running tap water. The washed samples were allowed to air dry. The air dried roots and leaves were cut into 5 mm^2 sized slices using sterilized scissors. The slices were then surface disinfected using 2% sodium hypochlorite (NaOCl) or 70% ethanol for 5 minutes (Trikarunasawat, 2008). The slices were washed three times with sterile distilled water to remove the disinfectant. Infected cotton roots, leaves and stem were used for isolation of fungal pathogens by using tissue-transplanting technique (Trikarunasawat, 2008). All the inoculated plates were kept in an incubator, at 25°C for 7 days.

4.4.1 Culture re-isolation and purification

The isolates were designated systematically with an acronym AAUFcot, meant to stand for Addis Ababa University *Fusarium* isolated from cotton. The cultures were periodically checked for mold growth starting from the 24th hour after inoculation and emerging mycelial hyphae were transferred to PDA medium by means of hyphal tips transfer technique for purification (Leslie and Summerell, 2006). From the PDA cultures a portion of the pure isolate was sub cultured to other PDA plates or slants and stored in refrigerator at 4°C.

4.5 Morphological Identification of *Fusarium* isolates

The isolates were grown on PDA plates at 25°C for 4 and 7 days. The observation was made in colony color or pigmentation, presence or absence of macroconidia microconidia, phialides and *chlamydospores*. Measurements were done in the radial growth of the isolates using millimeter calibrated ruler. Slide cultures for the pathogenic *Fusarium* isolates were prepared (Aneja, 2005). Morphological species identification was done by referring to the illustrative literature (Gerlach and Nierenberg, 1982; Leslie and Summerell 2006). Observation of macroconidial and

microconidial shapes and septation was made using compound microscope, at 400X magnification.

4.6 Pathogenicity test

4.6.1 Preparation of spore suspension

Pathogenicity of one *Fusarium* isolate that representatives from the3 selected sites were tested on detached cotton leaves that have grown for three weeks. The suspension of conidia of each isolate was prepared by scraping mycelia from 7 day's old culture, mixed in30ml of sterile distilled water and stirred vigorously for 90 sec and then filtered through two-layer cheese cloth. The concentration of spore suspension was adjusted to 1×10^5 spores/ml by using haemocytometer before inoculation.

4.6.2 Inoculation to detached leaves and greenhouse seedlings

Healthy leaves were collected from cotton growing in pot in the greenhouse, washed and surface-sterilized using 5% sodium hypochlorite solution for 30 Sec 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 tissue papers. The leaves were sprayed with spore suspensions of each isolate and incubated at 25°C until typical symptoms of *Fusarium* wilts were observed (Miller *et al*., 2011).

4.6.3 Re-isolation of isolated fungal pathogens

The causative agent in the diseased leaf parts was re-isolated on Potato Dextrose Agar (PDA). The characteristics of the re-isolates were compared with that of the original parent culture.

4.7 *In Vitro* evaluation of *Trichoderma* against *Fusarium* wilt of cotton

The antifungal activities of isolated endophytic fungi against the Fusarium isolates were evaluated by three different methods. These are dual culture and volatile and nonvolatile metabolite bioassays. Each treatment has replicated three times.

4.7.1 Dual Culture Method

The dual culture technique is used to assay the antagonistic activity of endophytic fungal isolates. A total of four *Trichoderma* isolates (T131, T136, ChenT14 and GimT7)was isolated from plant tissues (ChenT14 and GimT7) and soil samples (T131, T136) to evaluate their antagonistic potential against *Fusarium* wilt pathogen isolate. A plug of 5mm^{2}diameters from the edge of an actively growing was placed at the periphery of the culture plate and incubated for 4 days at 25°C. The plate were then inoculated with a 5mm^2 diameter mycelial disc of the endophytic isolate, placed 5cm from the pathogen at the opposite side, and incubated at 25°C. A

plate inoculated with *Fusarium*alone served as a control. Each treatment had three replicates. The growth inhibition rate was calculated.

4.7.2 Growth Inhibition Analysis

The *Fusarim* mycelial colony growth (mm) data were recorded at 2-day intervals. A final observation on radial mycelial colony growth were made after 10 days, when the presence of overlapping fungi on any one Petri dish was recorded. The growth inhibition rate (GI) was calculated using the following formula given by WhippsJM.(1997).

Formula: $$GI = \frac{R1 - R2}{R1} \times 100$$

Where: GI= growth inhibition rate

R1= radial growth of the pathogen without *Trichoderma* spp.

R2= radial growth of the pathogen with antagonistic *Trichoderma* spp.

4.7.3 Volatile Metabolite Inhibitory Bioassay

The effects of volatile antibiosis of antagonisticfungi on Fusarium were tested in the assemblage described by Dennis and Webster (1971) and Naraghi *et al*. (2010). A $5mm^2$ disc of *Fusarium* was placed in Petri dishes containing PDA medium and incubated at 25^oC for 4 days. Then, 5 mm^2 discs of endophytic fungi were also cultured in Petri dishes containing PDA medium. Two Petri dish bottoms (without their lids) containing pathogen and antagonist isolate were placed face to face and then sealed with thin plastic film. The control Petri dishes were not inoculated with antagonistic fungi. The Petri dishes were incubated at 25^oC for 10 days.

4.7.4 Nonvolatile Antibiotic Inhibitory Assay

The effect of accumulation of nonvolatile antibiotics produced by antagonistic fungi on *Fusarium* growth was examined using the exudate filtering method Whipps and Magan (1987). Filtrate extracts of the antagonistic fungi (T131, T136, ChenT14 and Gimt7) were mixing properly to the Petri dishes containing PDA medium by volume-volume ratio of 4ml:16ml respectively and centrally inoculated with $5mm^2$ discs of *Fusarium* isolate. Each treatment has three replicates. In the control plates 4ml distilled sterile water was added instead of the *Trichoderma* filtrates to 16ml PDA medium and *Fusarium* isolate were inoculated. The plates were incubated for 10 days at 25^oC and radial growths were measured in two days interval.

4.8 Preparation of potato dextrose broth (PDB) media with different pH values

Potato Dextrose Broth media (1600ml) was prepared and poured into 16 sterilized flasks which measure 100ml each. The pH of each 100 ml of PDB medium was adjusted to 4.5, 5.5, 6.5 and 7.5 by adding NaOH (5N) and HCl (1N) and checked by pH meter.

4.8.1 Sporulation of *Trichoderma* species at different pH values

To determine the rate of sporulation of the *Trichoderma* sp., at different pH values, a 5 mm diameter mycelial disc cut from the margin of a 7 day old *Trichoderma* (T131, T136, ChenT14 and GimT7) were innoculated. The innoculation was placed into potato dextrose broth medium flasks with different pH values (pH 4.5, 5.5, 6.5 and 7.5). There were 3 replicate flasks for each pH value. The flasks were incubated at room temperature (25°C) for 15 days. To measure the biomass of spores produced at each pH value and each *Trichoderma*. Spore suspension of 15 days old was poured into another sterilized flask using a filter paper. The remaining mycelia on the filter paper were dried in an oven to measure the dry weight. The poured culture filtrates were examined under spectrophotometer to measure the spore concentration.

4.8.2 Effect of temperature on the isolated *Trichoderma* sp.

To determine the rate of sporulation of the *Trichoderma* spat different temperature level, a 5 mm diameter mycelial disc was cut from the margin of a7day old *Trichoderma* (T131, T136, ChenT14 and GimT7) and each disc was placed in the center of PDA plates. There were three replicate plates for each *Trichoderma* isolate. The plates were incubated at temperature variations of (4°C), (25°C), and (37°C) for 10 days. The *Trichoderma* sp mycelial radial growth (mm) data were recorded at 2-day intervals.

4.9 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 Tukey's Honestly Significance Difference (HSD). The significance of effects of *Trichoderma* on growth characteristics was determined by the magnitude of the F value ($p < 0.05$).

5. RESULTS

5.1 Isolation of the *Fusarium* wilt pathogen from plant tissues

From sample specimens (leaves, stems and roots) of cotton, a total of 27isolates were obtained upon incubation at 25°C for 7 days. Six of these isolates (Fig-6) were resembled as the test pathogen (AAUFcot01, AAUFcot02, AAUFcot03, AAUFcot04, AAUFcot05 and AAUFcot06) and they were selected and subjected for further morphological characterization &identification.

5.2 Morphological Identification of *Fusarium* isolates

5.2.1 Cultural pigmentation

Based on cultural characters on PDA, the isolates showed different colony colors. Specifically, the colony color of the isolates on PDA medium varied from creamy white, fluffy white, grey, black, and brown (Fig-6). Based on the mycelial growth and color of the isolates, one isolate were identified as *Fusarium* isolates (Fig-7). Therefore, the identified *Fusarium* isolate (AAUFcot04) was selected and subjected to further pathogenicity and antagonistic tests.

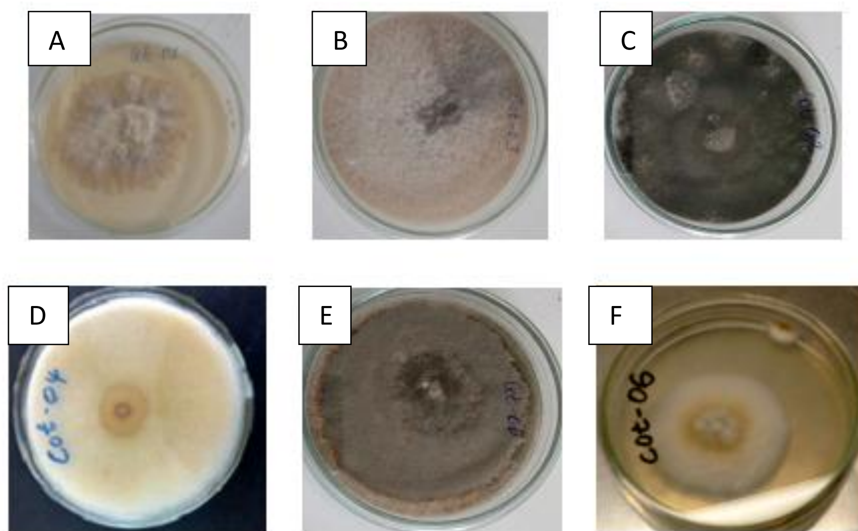

Figure 6: Six Fusarium isolates isolated from cotton samples: AAUFcot01 (A), AAUFcot02(B), AAUFcot03(C), AAUFcot04(D), AAUFcot05(E) and AAUFcot06(F).

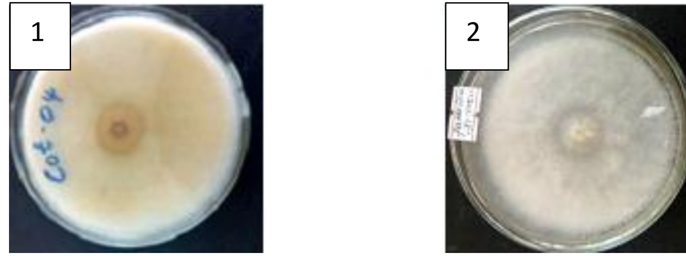

Figure 7: Colony color of the identified *Fusarium* isolates on PDA
(1- reverse side, 2- front side).

5.2.2 Slide culture and Microscopic features of *Fusarium oxysporum*

The results of slide culture and microscopic observation showed us various features such as size of microcroconidia and macroconidia were measured between the range of 80 micrometer and 360 micrometer (Fig-8). Similarly, an oval shaped microconidia and sickle shaped macroconidia were also observed clearly.

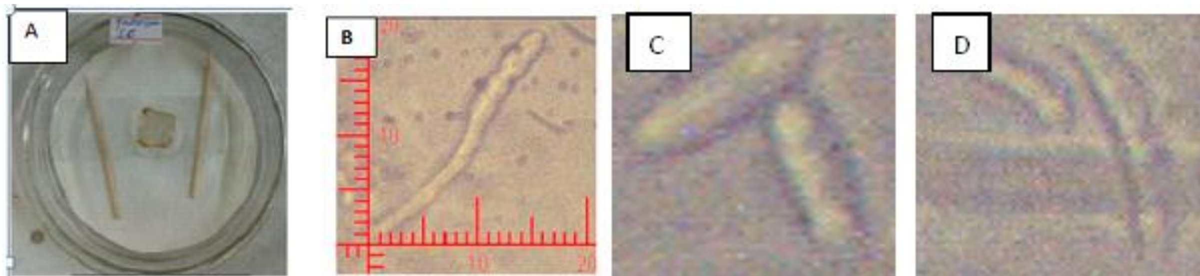

Figure 8 Microscopic features of *Fusarium* (A- slide culture preparation, B- size of macroconidia, C-oval shaped microconidia, D- sickle shaped macroconidia.

5.3 Pathogenicity test

Based on the results, yellow coloration and wilting symptoms were observed on the inoculated leaves of greenhouse seedlings. White hyphae growth on the petiole of detached leaves were also seen as a typical symptom of *Fusarium* wilt disease after inoculation with the test pathogenAAUFcot04 (Fig-8).

Figure 9: Pathogenicity test on detached leaves. 1- sources of cotton leaves, 2- disinfection process on the Biosafety, 3- inoculated detached leaf on a plate, 4- diseased leaf after incubation, 5- control pot, 6- diseased cotton plant.

5.3.1 Re-isolation of isolated fungal pathogens

The causative agent in the diseased leaf parts was re-isolated on potato dextrose agar (PDA). The characteristics of the re-isolates were compared with that of the original parent culture (Fig-9) accordingly; the re-isolated fungal pathogens were culturally and morphologically identical with that of the original parent culture.

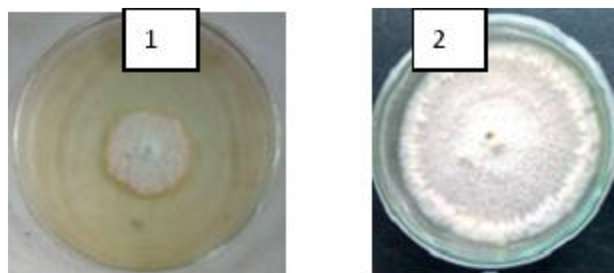

Figure 10: The re isolated culture of *Fusarium*: 1- four days old, 2-seven days old

5.4Growth Inhibition Analysis of dual culture antagonistic bioassay

Efficiently *Trichoderma* T131 showed growth inhibition with the highest (78.89%). On the other hand, least growth inhibition (52.29%) was caused by the *Trichoderma*GimT7 (Table - 3). In the

overall test of dual culture antagonism, all the *Trichoderma* isolates were significant at (P=0.05) against the pathogen.

Table 2: Evaluation of Effects of *Trichoderma* isolates on growth of isolated *Fusarium* species (AAUFcot04) in dual culture bioassay.

Trichoderma isolates	*Fusarium* isolate (AAUFcot04)		
	Day6	Day8	Day10
Chen-T14	62.16 ^{b}±4.68	67.41 ^{b} ±1.94	73.39 ^{a} ±1.58
Gim-T7	29.27 ^{c} ±6.19	41.57 ^{b} ±5.1	52.29 ^{b} ±4.2
T-136	64.86 ^{b} ±4.68	70.78^{ab} ±3.89	76.14 ^{a} ±3.17
T-131	68.91 ^{b} ±2.34	74.15 ^{a} ±1.94	78.99 ^{a} ±1.58
Total Mean ± Sd	52.07 ^{b} ± 22.42	58.59 ^{b} ±21.99	64.78 ^{b} ±22.2
Control	00±00	00±00	00±00

Mean ± standard diviation

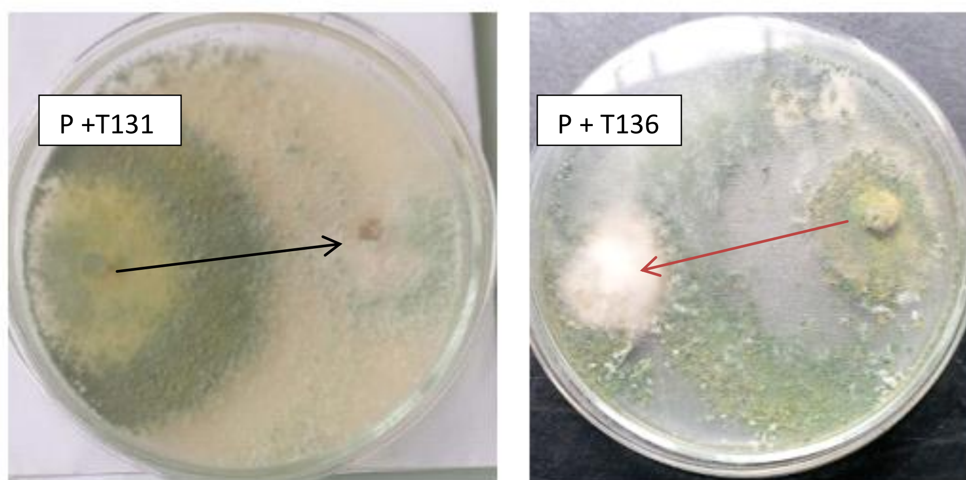

Figure 11: dual culture test plates

5.5Growth Inhibition Analysis of volatile and non-volatile metabolites bioassay

Trichoderma isolates produced volatile metabolites that significantly inhibited the growth of *Fusarium* species (Table-4). The volatile metabolites from the different isolates showed significant differences in the inhibition against the pathogen. The *Trichoderma* (T131) gave the highest inhibition 48.14%, followed by isolates T136 and ChenT14 at 47.40%, whereas the isolate GimT7 gave the least growth inhibition (34.07%) after 10 days of incubation (Table-4). Similarly the test was conducted using the non-volatile metabolite *Trichoderma*. Accordingly,

the highest percentage inhibition (52.89%) was gained by T136 and least growth inhibition (38.84%) was recorded on *Trichoderma* ChenT14 after 10 days incubation at 25°C.

Table 3: Evaluation of Effects of *Trichoderma* isolates on growth of isolated *Fusarium* species (AAUFcot04) in volatile and nonvolatile metabolite compounds

***Trichoderma* isolates**	***Fusarium* isolate (AAUFcot04)**					
	Volatile compounds			**Nonvolatile compounds**		
	Day6	**Day8**	**Day10**	**Day6**	**Day8**	**Day10**
Chen-T14	33.96^{b}±7.12	41.80^{b}±6.18	47.40^{b}±5.5	44.77 ^{b}±13.67	37.36 ^{b}±6.59	38.84 ^{b}±7.57
Gim-T7	16.98^{c}±18.8	27.04^{c}±16.7	34.07^{c}±15.1	44.77^{b}±11.26	34.06^{c} ±14.37	39.66 ^{b}±18.93
T-136	38.67^{b}±17.0	43.44 ^{b}±20.1	47.40^{b}±20.6	35.82^{b}±11.2	48.35^{b}±15.57	52.89^{ab}±13.1
T-131	33.96^{b}±18.2	42.62 ^{b}±15.8	48.14^{b}±14.2	32.83^{c} ±4.4	43.95 ^{b}±8.72	49.58^{ab}±3.78
Total Mean ± Sd	28.51^{b}±17.7	37.75^{b}±17.9	40.85 ^{b}±18.2	36.5 ^{b}0±15	37.78 ^{b}±15.95	41.76^{b}±17.2
Control	00±00	00±00	00±00	00±00	00±00	00±00

Means in the same column for each treatment followed by same letter(s) are not significantly different according to Tukey HSD. (P= 0.05).

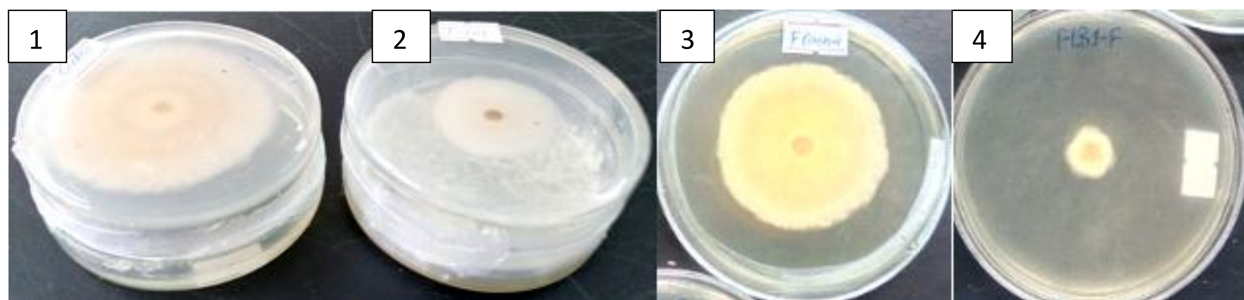

Figure 12 Antagonistic effect of volatile and non-volatile metabolites against the pathogen.1&3-control (AAUFcot04), 2&4- *Trichoderma* (T131) for volatile and non-volatile tests respectively.

5.6Growth analysis of *Trichoderma* species at different temperature level

From the results obtained in the growth analysis of *Trichoderma* isolate at different temperature level, most of them showed passive growth at 4°C (Fig-13). Based on the observation of the recorded results 25°C was the best one for the growth of the isolated *Trichoderma* species. However, among the isolates T131 was exceptionally suppressed to grow at high temperature (37°C).

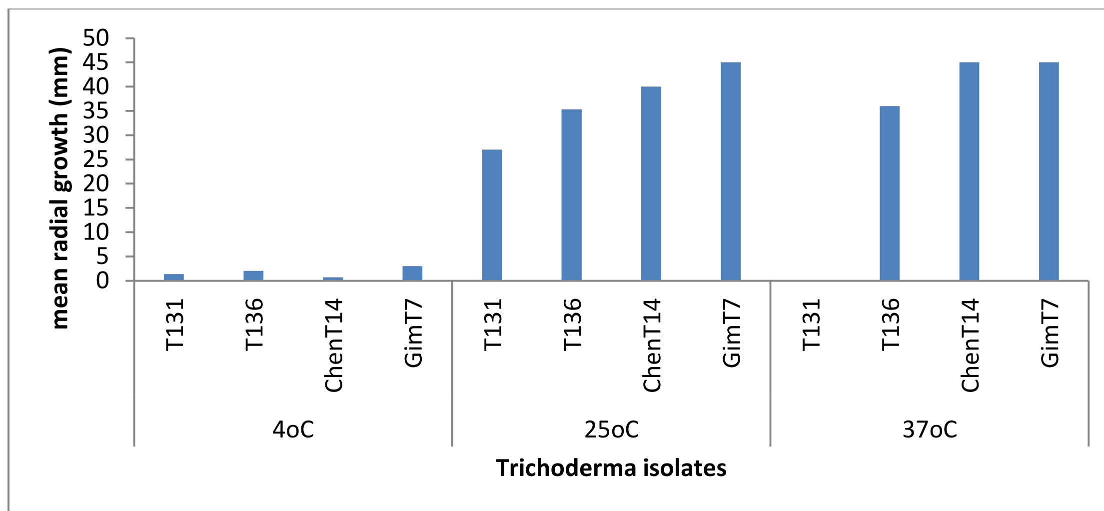

Figure 13 : mean radial growth of *Trichoderma* isolates at different temperature level.

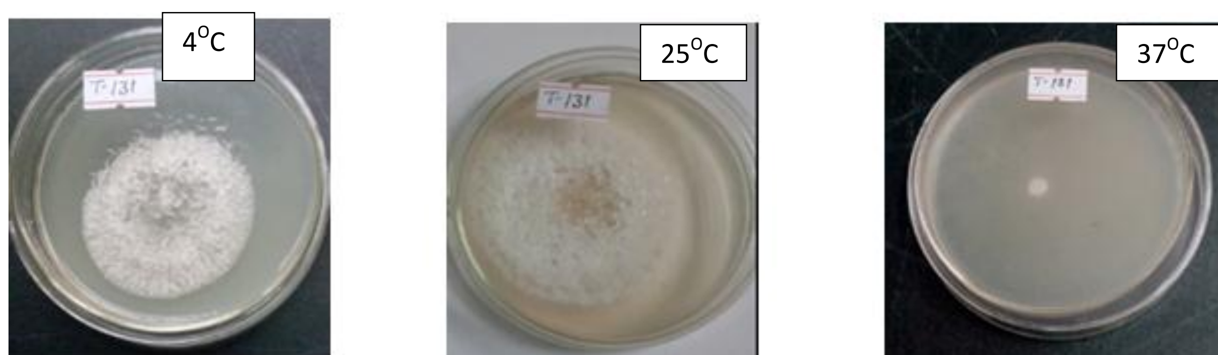

Figure 14 comparison on radial growth of particular *Trichoderma* (T131) at different temperature.

5.7 Sporulation of *Trichoderma sp*. at different pH values

Trichoderma T131 showed the highest dry mass (0.502g) at a pH of 6.5 (Table-5). On the other hand, the least dry mass (0.219 g) was obtained from the isolate T136 at a pH of 4.5. The absorption of light (OD reading) of the isolated *Trichoderma* species filtrates was also measured under spectrophotometer and then the *Trichoderma* GimT7 at pH 4.5 shown the highest absorption (1.23) followed by ChenT14 at a pH of 7.5 and T136 at pH 4.5 (0.718, 0.692 respectively), whereas least absorption (0.002) was seen by the *Trichoderma* T131 at a pH of 4.5. The pH change is not that significant except in *Trichoderma* isolate GimT7 of which change in pH shows more variation than other isolates.

Table 4: OD reading and dry mass of the experimental *Trichoderma* at a given pH level

Lists Of *Trichoderma*	**OD reading at pH**				**Dry mass (g) at pH**			
	4.5	**5.5**	**6.5**	**7.5**	**4.5**	**5.5**	**6.5**	**7.5**
T131	0.002	0.034	0.047	0.156	0.363	0.472	0.502	0.467
T136	0.692	0.32	0.617	0.492	0.219	0.308	0.267	0.34
ChenT14	0.019	0.026	0.05	0.718	0.399	0.22	0.494	0.301
GimT7	1.23	0.569	0.471	0.347	0.267	0.319	0.252	0.241

6. Discussion

Samples of the cotton plant tissues were collected from the study area and brought to the mycology laboratory at Addis Ababa university initial 27 isolates, six isolates resembled as *Fusarium* wilt causing pathogens. Through further purification and microscopic examination one isolate AAUFcot04 was identified as the target pathogen isolate of cotton crop.

Antagonistic effect based on the dual culture experiments showed that *Trichoderma* isolates significantly inhibited the mycelial growth of *Fusarium* species ranging from 52.29%-78.89% against *Fusarium* species after 10 days of incubation at 25°C (Table-3). However, all the *Trichoderma* isolates suppressed the mycelial growth of the test fungus in *in vitro* evaluation. Isolate T131 gave the highest inhibition percentage value of 78.89%. In similar studies, Susanto *et al*. (2005) have also documented that *T. harzianum* against *Drechsleratriticirepentis* gave the highest inhibition capacity by 97.8% in dual cultural analysis. And also,Perello*et al*. (2003) have reported that *Trichoderma* were significantly inhibited *Drechsleratriticirepentis* colony growth between 50 and 74% utilizing dual culture techniques on PDA.

Inhibitory effect due to the production of secondary metabolites by the *Trichoderma* isolates was also evaluated. *Trichoderma*. Produced both volatile and nonvolatile compounds that suppressed the growth of the fungal pathogens.The growth of *Fusarium* species was inhibited when exposed to the trapped headspace of volatile compounds produced by *Trichoderma* isolates. Unlike the results of the dual culture test, *Trichoderma* isolates GimT7 inhibited the growth of the pathogen fungus by 34.07%, which is the least inhibition of all the *Trichoderma* isolates. This may indicate that this isolate produced less effective volatile compound against the test pathogen. Whereas, isolates T131 produced active volatile compound which inhibited the growth of the *Fusarium* species by 48.14% (Table-4). These results are inconsistent with the study conducted by Siddiquee*et al*. (2009) that *Trichoderma* inhibited the mycelial growth of *G. boninense* up to 70%. Doi and Mori (1994) observed that volatile compounds from *Trichoderma* spp impeded the hyphal growth of different fungal pathogens on agar plates. It is also reported that *T. viride* produced large amounts of volatile compounds to affect the hyphal tips of *Lentinuslepidus* and *Coriolus versicolor*. Kucuk and Kivanc (2003) have observed that the volatile metabolites of *T. harzianum* isolates also have inhibitory effects on the growth of the plant pathogens tested.

The optimum temperature for growth differs among the *Trichoderma* isolates. In the present study, all the isolates of the *Trichoderma* grew best at a temperature of 25°C and 37°C and the

optimum temperature for all *Trichoderma* isolates was 25°C. Kredics*et al*. (2003) have also reported that the optimum temperature for the best growth of *Trichoderma* species was 25°C and 30°C. All the isolates T131, T136, ChenT14 and GimT7 had higher growth rates at 25°C (Fig-13). The isolates except T131 had also best growth rates at 37°C. The rate of growth of isolates at a relatively lower temperature 4°C was extremely slow. Kredics*et al*. (2003) have also reported that cold tolerant *T. aureoviride, T. harzianum*and *T. viride* grew well at 5°C. However, at the present study it was observed that the *Trichoderma* isolates showed limited growth as the temperature decreasedto 4°C.

Another important environmental factor that has an effect on the growth of the *Trichoderma* isolates is pH characteristics. pH has an effect on mycelial growth and mycoparasitism of *Trichoderma*. In the present study, the effect of pH on the mycelium growth of the isolates varied slightly among the isolates. Their mycelial dry weight revealed that *Trichoderma* grew well in the pH range of 4.5, 5.5, 6.5, and 7.5 with a slight variation of dry weight (Table-5). Isolate T131 performed better in comparison with other isolates which has shown the maximum mycelial dry weight at a pH value 6.5. This may indicate that they are less resistant to strong acidic conditions. The optimum pH for maximum biomass production is at 5.5-7.5. Similarly,Kredics*et al.* (2003) have reported that the optimum pH range for maximum growth of *Trichoderma* species was pH 5.5-7.5.

Sporulation is an important characteristic of biocontrol agents as their efficiency and competence of biocontrol is closely associated with the ability to compete with pathogens in the soil. In the present study the highest sporulation (1.23) was recorded the*Trichoderma* isolate (GimT7) at a relatively low pH 4.5 (Table-5). Carreras-Villasen*et al.* (2012) have reported that sporulation of *Trichoderma* is a low pH dependent process and *T. harzianum* shows the highest sporulation at pH 5.5. The studies of Ali *et al*. (2015) and Zehra *et al*. (2014) confirmed that the pH 6 was the best for the growth and sporulation of different *Trichoderma* spp. under laboratory conditions, which is in agreement with the observations of the present study. Furthermore, Bandyopadhyay *et al*. (2003) and Singh *et al*. (2014) have reported that *Trichoderma* isolates exhibit optimum growth and sporulation rate at pH values ranging from 2 to 7. The suitability of an acidic pH ranges for the survival of *Trichoderma* was also reported by Bhai *et al*. (2010) where they observed that the pH range 4.5–5.5 was suitable for the growth, sporulation, and survival of *Trichoderma* than alkaline conditions under greenhouse conditions.

7. Conclusions and Recommendation

7.1 Conclusions

- The four *Trichoderma* isolates evaluated, performed well as biocontrol agents when tested individually with *Fusarium* species on culture medium. Hence, T-131 was especially potential biological control agent to suppress the cotton wilt pathogen in this study.
- The pH value ranging from 5.5-7.5 was relatively optimum for mycelial growth of *Trichoderma* isolates according to dry weight measurements and pH values 4.5-6.5 was optimum for sporulation of *Trichoderma* isolates measured as OD readings.
- The optimum temperature for the best growth of *Trichoderma* was 25°C. Mycelial growth of *Trichoderma* isolates was found to be limited as temperature reduced to 4°C.
- Evaluation the mycelial growth of the pathogenic fungus was inhibited by the production of volatile and nonvolatile compounds of *Trichoderma* isolate under laboratory. Accordingly, T 131 and T 136 showed better performance in growth inhibition of the test pathogen isolate

7.2 Recommendations

- *Invivo* testing and evaluation of the four *Trichoderma* isolates to be well studied in the future. Especially the T-131 would be promising biological control agent in managing the cotton wilt disease in the study areas.
- The molecular identification of the isolated pathogen and the *Trichoderma* isolates would be beneficiary in the future studies.

8. References

Abd-Elsalam, KA., Omar, MR., Migheli, Q., Nirenberg, HI. (2004). Genetic characterization of *Fusariumoxysporum*f. *Sp.Vasinfectum* isolates by random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). *Journal of Plant Disease and Protection,***111**:534-544.

Abd-Elsalam, KA., Omar, R., Asran-Amal, A., Aly, A.A. (2009). Differential interaction between cotton genotypes and isolates of *Fusarium oxysporum*f. *Sp.vasinfectum*. *Archive of Phytopathology and Plant Protection,***42**:464-473.

Abo, K., Klein, K.K., Edel-Hermann, V., Gautheron, N., Traore, D., Steinberg, C. (2005). High genetic diversity among strains of *Fusarium oxysporum*f. *Sp.vasinfectum*from cotton in Ivory Coast. *Phytopathology,***95**:1391-1396.

Agrios, G.N. (1997). *Plant Pathology,* 4th edition. Academic Press, Inc: New York. Pp.635.

Agrios, G.N. (2005). *Plant Pathology*, 3rd ed. Academic Press, Inc: New York. Pp.840-948.

Aguiar, F.M., Michereff, S.J, Boiteux, L.S, Reis, A. (2013). Search for sources of resistance toFusarium wilt (*Fusarium oxysporum*f.sp. *vasinfectum*) in okra germplasm. *Crop Breeding and Applied Biotechnology,***13**:33-40.

Aktar, W., Sengupta, D. and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology,***2:** 1-12.

Ali, H.Z., Aboud, H.M., Dheyab, N.S., Musa, N.K. &Gasam, F.H. (2015). Effects of pH and ecw on growth and sporulation of indigenous Trichoderma spp. *International Journal of Phytopathology,* **4**(**1**): 15–20.

Aly, A.A., Hussien, E.M., Allam, A.D.A., Amein, A.M. and El-Samawaty, A.M.A. (2000). Pathological studies on fungi involved in damping-off of cotton seedlings and root rot of adult plants in upper Egypt governorates. *J. Agric. Sci*. **25**: 4015-4034.

Amadou, C., Pascal, G., Mangenot, S., Glew, M., Bontemps, C., Capela, D., Carrere, S., Dossat, C., Lajus, A., Marchetti, M., Poinsot, V., Rouy, Z., Servin, B., Saad, M., Schenowiyz, C., Barbe, V., Batuit, J., Medigue, C., Masson-Boivin, C. (2008). Genome sequence of b-rhizobium Cupriavidustaiwanensis and comparative genomics of rhizobia. *Genome Res.***18**:1472–1483

Aneja, K.R. (2005). *Experiments in Microbiology, Plant Pathology and Biotechnology*. New Age Publishers, New Delhi, pp. 69.

Ashour, N.I., and A.M. Abd-El'Hamid. (1970). Relative salt tolerance of Egyptian cotton varieties during germination and early seedlings development. *Plant and Soil,***33**: 493-495.

Ashraf, M. (2002). Salt tolerance of cotton: Some new advances. *Critical Reviews in Plant Sciences,* **21**: 1-30.

Atkinson, GF. (1892). Some diseases of cotton. *Bulletin Alabama Agricultural Experiment Station,* **41**:19-29.

Baker, E. F. and Cook, R. J. (1974). *Biological control of plant pathogens*. W.H. Freeman & Co. Sanfransisco. Pp. 433.

Baker, K. F. (1987). Evolving concepts of biological control of plant pathogens. *Annu. Rev. Phytopathol*, **25**:67-85

Bandyopadhyay, S., Subhendu, J. & Dutta, S. (2003). Effect of different pH and temperature levels on growth and sporulation of *Trichoderma.Environmental Ecology,***21**: 770–773.

Beckman, C.H. (1987). *The Nature of Wilt Diseases of Plants*. APS Press, St. Paul.

Benítez, T., Rincón, A.M., Limón, M.C. &Codón, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology,***7:** 249–260.

Ben-Yephet, Y., Stapleton, J. J., Wakeman, R. J. and DeVay, J. E. (1987). Comparative effects of soil solarization with single and double layers of polyethylene film about survival of *Fusarium oxysporum f. Sp. vasinfectum*. *Phytoparasitica,* **15**:181-185

Bhai, R.S., Raj, S. & Kumar, A. (2010). Influence of soil pH and moisture on the biocontrol potential of *Trichoderma harzianum* on Phytophthora capsici - black pepper system. *Journal of Biological Control,***24** (2): 153–157.

Bilgram, K.S. and Dube, H.G. (1976). Modern plant pathology.Bond, D.A. (1979). English names of Viciafaba broad bean, field bean or faba bean. FABIS - Newletter 1-15.*BioEssays,* **12**: 53–59.

Boonk, W., De Geer, D., Kreek, E., Remme, J. and Huystee, B. (1998). Itraconazole in the treatment of tinea corporis and tinea cruris: comparison of two treatment schedules. *Mycoses,***41**: 509-514.

Booth, C. (1971). The genus *Fusarium*. Kew UK. Common wealth Mycological Institute.n Chen, Q., Ji X, Sun, W. (1985) Identification of races of cotton wilt Fusarium in China. *Journal of Agricultural Sciences,***6**:1-6.

Brubaker, C.L., F.M., Bourland and J.F. Wendel. (1999). *The origin and domestication of cotton.* Pp. 3-31 **In**: W.C. Smith and J.T. Cothren, eds., Cotton: Origin, History, Technology and Production. John Wiley & Sons, New York.

Burgess, LW., Trimboli, D. (1994). Characterisation and distribution of *Fusarium*nygamai, sp. nov.*Mycologia,***78**: 223-9.

Carreras-Villasen, N., Sa´nchez-Arreguı´n, JA. & Herrera-Estrella, AH. (2012). *Trichoderm*a: sensing the environment for survival and dispersal. *Microbiology,* **158**: 3–16.

Christiansen, M.N., Moore, R.P. (1959). Seed coat structural differences that influence water uptake and seed quality in hard seed cotton. *Agronomy Journal,* **51**: 582-584.

Costa, M.L.N., Dhingra, O.D. and Da-Silva, J.L. (2005). Influence of internal seed bore *Fusarium semitectum* on cotton seedlings. *Fitopatologia Brasileira.***30**:183-186.

Cothren, J.T. (1999). *Physiology of the cotton plant*. Pp. 207-268 in W.C. Smith and J.T. Cothren, eds., Cotton: Origin, History, Technology and Production. John Wiley & Sons, New York.

Coutinho, E.M. (2002). Gossypol: A contraceptive for men. *Contraception,* **65**: 259-63.

Davidson, T. R., and Townshend, J. L. (1967). Some weed hosts of the southern root-knot nematode, Meloidogyne incognita. *Nematologica,* **35**:452-458.

Davis, R.M., Colyer, P.D., Rothrock, C.S., Kochman, J.K. (2006). Fusarium wilt of cotton: population diversity and implications for management. *Plant Disease,* **90**: 692-703.

Davis, RD., Moore, NY., Kochman, JK. (1996). Characterization of a population of *Fusarium oxysporum*f. sp. *vasinfectum*causing wilt of cotton in Australia. *Australian Journal of Agricultural Research,* **47**:1143-1156.

De Vrije, T., Antoine, N., Buitelaar, R.M., Bruckner, S., Dissevelt, M., Durand, A., Gerlagh M., Jones, E.E., Luth, P., Oostra, J., Ravensberg, W.J., Renaud, R., Rinzema, A., Weber, F.J. and Whipps, J.M. (2001). The fungal biocontrol agent Coniothyriumminitans: Production by solid-state fermentation, application and marketing. *Applied Microbiology and Biotechnology*. **56**: 58-68.

Dennis, C., Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma.Br. Mycol. Soc*. **57**: 25-39.

Doi, S. and Mori, M. (1994). Antifungal properties of metabolites produced by Trichoderma isolates from sawdust media of edible fungi against wood decay fungi. *Material Organism,***28**:143-151.

Edel, V., Steinberg, C., Gautheron, N., Alabouvette, C. (2000). Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. *Mycol.Res*. **104**: 518–526.

Elad, Y., Chet, I. & Katan, J. (1980). *Trichoderma*harzianum: A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani. *Phytopathology,***70**(2): 119–121.

El-Samawaty A.M.A. (2004). *Pathological studies on the interaction between some Fusarium spp. and cotton plants*. Ph.D. dissertation, MiniaUnvi., Minia, Egypt.

El-Samawaty, A.M.A., Abdel-Reheem, M.A.T., Abd-Esalam, K.A. and Omar M.R. (2008). Use of random amplified polymorphic DNA (RAPD) to differentiate among isolates of *Fusarium spp*. Pathogenic on cotton. *J Biol Chem Environ Sci*. **3**(1):811-827

Ethiopian Investment Agency. (2002*). Investment Opportunity Profile for Cotton Production and Ginning in Ethiopia*

Ethiopian Investment Agency. (2012). *Investment Opportunity Profile for Cotton Production and Ginning in Ethiopia*

Fahmy T. (1927). The *Fusarium* wilt disease of cotton and its control. *Phytopathology,* **17**:749-767.

Fassihiani, A. (2000). Symptomless carriers of the causal agent of tomato wilt pathogen. *Journal of Agriculture, Science and Technology*, **2**: 27-32.

Feng, J., Wen, S., Leiyan, S., Ma, C., Feng, J., Sun, WJ., Shi, CLY. (2000). RAPD analysis of physiologic races of *Fusarium oxysporum*f. sp. *vasinfectum*in China. *Mycosystema,***19**:45-50.

Fourie, G., Steenkamp, E.T., Gordon, T.R., Viljoen, A. (2009). Evolutionary relationships among the vegetative compatibility groups of *Fusarium oxysporumf.spcubense*. *App*l. *Environ. Microb*. **75**: 4770–4781.

Fravel D. (2005). Commercialization and implementation of biocontrol. Annu. Rev. *Phytopathol.***43**: 337-359.

Fravel D.R., Connick W.J. and Lewis J.A. 1998. *Formulation of microorgansisms to control plant diseases.***In**: Burges HD, ed. Formulation of Microbial Biopesticides. Dordrecht: Kluwer Academic Publishers, 187-202.

Fryxell, P.A. (1992). A revised taxonomic interpretation of *Gossypium* L. (Malvaceae.) *Rheedea,* **2**:108-165.

Galal, H.E., Abou-el-fittouh, H.A., Morshed, G. (1972). Effect of direction and distance on cross pollination in Egyptian cotton (Gossypium barbadense L.). *Experimental Agriculture,* **8**: 67-71.

Gardiner, DM., Kazan, K., Manners, JM. (2013) Cross-kingdom gene transfer facilitates the evolution of virulence in fungal pathogens. *Plant Sci.*, **210**:151–158

Geiser, D.M., Jimenez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Kuldau, G.A. and O`Donnell, K. (2004). FUSARIUM – ID V. 1.0: A DNA sequence database for identifying *Fusarium*. *European journal of plant pathology,***110**: 473-479.

GeremewTerefe. (1990). *The status of cotton diseases in Ethiopia*. Proceedings of the 15th annual meeting of the Ethiopian phytopathological committee. IAR, Addis Ababa, Ethiopia.

Gerlach, W. and Nirenberg, H. (1982). *The Genus Fusarium- a Pictorial Atlas*. "Institut fur Mikrobiologie", Berlin, pp. 345-350.

Govila, O.P. (1969). Fertilization and seed development in crosses between *G.arboreum*and*G. hirsutum*. *Indian J. Genetics*, **29**: 407-417.

Govila, O.P. and Rao, C.H. (1969). Studies on the in vitro germination and storage of cotton pollen. *Journal of Palynoloy*. **5**: 37-41.

Guitchount, A. (2003). *The structure of world trade*. Washington DC, ICAC.

Hall, F.R. and Menn, J.J. (1999). *Biopesticides*: Use and Delivery. Totowa, NJ: Humana Press.

Handelsman, J. (1996). Biocontrol of soilborne plant pathogens. *Plant Cell,* **8**:1855-1869.

Harman, G. (2000). Myths and dogmas of biocontrol: changes in percetions from research on *Trichoderma* harzianum T-22. *Plant Dis*. **84**:377-393

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

Harman, G., Kubicek, C. (1998). *Trichoderma and Gliocladium*. Vol. II. Taylor and Francis, London, pp. 153–171.

Hermosa, R., Rubio, MB., Cardoza, RE., Nicolás, C., Monte, E. & Gutiérrez, S. (2013). The contribution of *Trichoderma* to balancing the costs of plant growth and defense. *International Microbiology,***16**(2): 69–80.

Hillocks, R. J., and Kibani, T.H. (2002). *Factors affecting the distribution, incidence and spread of Fusarium wilt of cotton in Tanzania.Experimental Agriculture* 38, 13 History,Technology and Production. John Wiley and Sons, Inc., pp 435-449.

Hillocks, R.J. (1992). *Fusarium wilt*. In: Hillocks R.J. (Ed.) *Cotton diseases. Melksham UK. Redwood Press* Ltd. pp. 127-160.

ICAC, (2003). *Assessment of the impact and main dynamics of cotton diseases affecting in particular small-scale production systems in Southern and Eastern Africa*. http://www. icac.org/projects/CommonFund/seacf_disease/proj_11_final.pdf.Accessed25th December, 2013.

Inoue, I., Namiki, F. and Tsuge, T. (2002). Plant colonization by the vascular wilt fungus Fusarium oxysporum requires FOW1, a gene encoding a mitochondrial protein. *Plant Cell*, **14**:1869-1883.

Isaacman, Allen and Richard, Roberts. (1995). *Cotton, Colonialism, and Social History in Sub-Saharan Africa*. London.

Isaacman. (1996). *Strategic Framework for the Development of the Cotton Sector in Burkina Faso.*Government of Burkina Faso, Burkina Faso.

Jackson, M.A. (1997). Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. *Journal of Industrial Microbiology & Biotechnology*. **19**: 180-187.

Jimenez-Diaz, R.M., Jimenez-Gasco, M.M. (2011). *Integrated management of Fusarium wilt diseases.***In** Alves-Santos, F.M., Diez, J.J. (Eds.), Control of Fusarium Diseases. Research signpost, Kerala, India, pp.177 – 215.

Jorgenson, E. C., Hyer, A. H., Garber, R. H. and Smith, S. N. (1978). The influence of soil fumigation on the Fusarium root-knot nematode complex of cotton in California. *Journal of Nematology*. **10**:228-231.

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

Karaosmano, lu., F., M., Tüter, E., Göllü, S., Yanmaz and E., Altinti. (1999). Fuel properties of cottonseed oil. Energy Sources, Part A: *Recovery, Utilization, and Environmental Effects,* **21**: 821-828.

Kok, L. and Victoria, K. (1999). *Biological Control for the Public*. (*http://www.biocontrol. ento.vt.edu*)

Kontoyiannis, B., Lee, S. and Taylor, J. W. (2000). Genetic Diversity in the Plant-Pathogenic Fungus Fusarium oxysporum. *The American Phytopathological society*. **10**: 475 - 874.

Kredics, L., Antal, Z., Anczinger, L., Szekeres, A., Kevei, F & Nagy, E. (2003). Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. *Food Technology and Biotechnology,* **41**(1): 37–42.

Kucuk, C. and Kivac, M. (2003). Isolation of Trichoderma Spp. and determination of their antifungal, biochemical and physiological features. *Turk J Biol*. **27**: 274-253

Kuhn, D.N., Cortes, B., Pinto, T., Weaver, J., (1995). Parasexuality and heterokaryosis in *Fusarium oxysporumf.sp. cubense*. *Phytopathology,***85**: 1119.

Larkin, R.P., and Fravel, D.R. (2002) *Phytopathology*, **92**:1160.

Lee, J.A. (1984). Cotton as a world crop. Pp. 1-25 in R.J. Kohel and C.F. Lewis, eds., Cotton. *Agronomy Monograph* No. 24. ASA, CSSA and SSSA, Madison, Wisconsin, USA.

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

Lucas, J.A. (1998). *Plant pathology and plant pathogens*, 3rd edn. Blackwell Science, p 274

Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trail, F., Gardiner, D.M., Manners, J.M., Kazan, K. (2013) *Fusarium* pathogenomics. *Annu Rev Microbiol.* **67**:399–416

Mace, M.E., Bel, A.A. and Beckman, C..H. (1981). *Fungal Wilt Diseases of Plants*. Academic Press New York and Lond.

Marasas, W. F. O., Nelson, P. E and Toussoun, T. A. (1984). *Toxigenic Fusarium species*:Identity and Mycotoxicology. The Pennysylvania State University Press.*um*.

Mayayo, E., I. Pujol, and J. Guarro. (1999). Experimental pathogenicity of four opportunist *Fusarium* species in a murine model. *Journal of Medical Microbiology*, **48**:363366.

McClure, T.T. (1949). Mode of infection of the sweet potato wilt *Fusarium phytopathology,***39**: 876-886.

McDonald, B.A. (1997). *The population genetics of fungi*: tools and techniques.

McDonald, B.A., Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol*, **40**: 349–379.

McDonald, B.A., McDermott, J.M. (1993). Population genetics of plant pathogenicfungi. *BioScience,***43**: 311–319.

Menzenburg, R.L., Glass, N.L. (1990). *Mating type and mating strategies in Neurospora*.

Miller, S.A., Rowe, R.C. and Riedel, R.M. (2011). *Fusarium and Verticillium wilts of tomato, potato, pepper, and eggplant. Fact Sheet*. Columbus, Ohio State University

Minton, N. A. (1986). Impact of conservation tillage on nematode populations. *Journal of Nematology,***18**:135-140.

Mitter, B., Brader, G., Afzal, M., Compant, S., Naveed, M., Trogitnz, F., Sessitsch, A. (2013) *Advances in eluciadating beneficial interaction between plant soil and bacteria*. In: Sparks DL (ed) *Advances in agronomy*, vol **121**. Elesvier, San Diego, pp 381–445

Moretti, A. (2009). Taxonomy of Fusarium genus, a continuous fight between lumpers and splitters. *Proc. Nat. Sci, Matica Srpska Novi Sad*. **117**: 7-13.

Morrell, J.J. and Bloom, J.R. (1981). Influence of Meloidogyne incognita on Fusarium wilt of tomato at or below the minimum temperature for wilt development. *Journal of Nematology,***1**(1): 57-60.

Mueller, W.C. and Beckman C. H. (1988). Correlated light and electron microscope studies of callose deposits in vascular parenchyma cells of tomato plants inoculated with Fusarium oxysporumf .splycopersici. *Physiological and Molecular Plant Pathology.***33**: 201-208.

Mulat, D.,Tewodros, N., Solomon, D., Assefa, B., and Temesgen, A. (2004). *Decent Work deficits in the Ethiopian Cotton Sector. October 2004. [Online] Available from: http://www.ilo.org/public/english/regional/afpro/addisababa/sro/pub/cottoncasestudy.pdf . [Accessed on 10 October 2007]*

Mustafa, M.H. (1983). *Root rot/wilt complex, powdery mildews, and Mosiac Diseases of Faba bean and their control.* P. 119.in M.C. Saxena and S. Varma (eds) International Workshop 16-20 May 1983. Faba beans, Kabule chickpeas, and Lentils in the 19080s.

Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., Afshari-Azad, H. (2010). Biological control of *Verticillium* wilt of greenhouse cucumber by Talaromyces flavus. *Phytopathol. Mediterranea,***49**: 321-329.

Nehl, D.B., Allen, S.J., Mondal, A.H. and Lonergan, P.A. (2004). Black root rot: A pandemic in Australian cotton. *Australas. Plant Pathol*. **33**:87-95.

Nelson P., Toussoun T. and Marasas W. F. O. (1983). *Fusarium* species: an illustrated manual for identification. University Park, Pennsylvania: Pennsylvania State University Press. pp. 193.

Nelson, P.E. (1991). *History of Fusarium systematics. Phytopathology,***81**, 1045–1048.

Nelson, P.E., Tousson, T.A. and Cook, R.J. (1981). *Fusariumi Diseases, Biology and Taxonomy*.The Pennsylvania State.Univ. Press, U.S.A.; 365-366.

O'Brien, R.D., L.A. Jones., C.C. King., P.J. Wakelyn and P.J. Wan. (2005). Cottonseed oil. Pp. 173-279 **In**: Bailey's Industrial Oil & Fat Products, 6th ed. (F. Shahidi, ed.), Vol. **2**, *Edible Oil and Fat Products*: Edible Oils (Part 1). John Wiley & Sons, Hoboken, New Jersey, USA.

O'Donnell, K., Gueidan, C., Sink, S., Johnston, P.R., Crous, P.W., Glenn, A., Riley, R., Zitomer, N.C., Colyer, P., Waalwijk, C. (2009) A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genet Biol*.**46**:936–948

O'Donnell, K., Sutton, D.A., Fothergill, A., McCarthy, D., Rinaldi, M.G., Brandt, M.E., Zhang, N., Geiser, D.M. (2008). *Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the Fusarium solani species complex. J. Clin. Microbiol.* **46**: 2477–2490.

OGTR. (2002). *The Biology and Ecology of Cotton (Gossypium hirsutum) in Australia*. Office of theGene Technology Regulator (OGTR), Canberra, Australia. <http://www.ogtr.gov.au/pdf/ir/biologycotton.pdf>

Oosterhuis, D.M., and J. Jernstedt. (1999). *Morphology and anatomy of the cotton plant*. Pp. 175-206 **In:**W.C. Smith and J.T. Cothren, eds., Cotton: Origin, History, Technology and Production. John Wiley & Sons, New York.

Palmateer, A.J., McLean, K.S., Morgan-Jones, G. and Van Santen, E. (2004). Frequency and diversity of fungi colonizing tissues of upland cotton. *Mycopathol*. **157**:303-316.

Perello, A., Monaco, C., Simond, M., Sisterna, M. and Bello, G. (2003). Biocontrol efficacy of Trichoderma isolates for tan spot of wheat in Argentina. *Crop Prot*. **22**:1099–1106.

Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krska, R., Kuchler, K., Glössl, J., Luschnig, C., Adam, G. (2003). *Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from Arabidopsis thaliana. J Biol Chem.***278**:47905–47914

Proctor, R.H., Butchko, R.A.E., Brown, D.W., Moretti, A. (2007). Functional characterization, sequence comparisons and distribution of a polyketide synthase gene required for perithecial pigmentation in some *Fusarium* species. *Food Addit Contam.***24**:1076–1087

Randey, B.P. (1986). *The fungi a textbook of Botany New Delhi, 4thedition* pp. 275-277.

Raza, W., Faheem, M., Yousaf, S., Rajer, F.U &Yameen, M. (2013). Volatile and non-volatile antifungal compounds produced by *Trichoderma* harzianum SQR-T037 suppressed the growth of *Fusarium oxysporum f. sp. niveum*. *Science Letters,***1**(1): 21–24.

Reddy, K.R. and H.F. Hodges. (2006). Exploring the limitations for cotton growth and yield. *Journal of New Seeds,***8**(2): 1-22.

Sarrocco, S., Mikkelsen, L., Vergara, M., Jensen, D.F., Lubeck, M. and Vannacci, G. (2006). Histopathological studies of sclerotia of phytopathogenic fungi parasitized by a GFP transformed *Trichoderma virens* antagonistic strain. *Mycological Research*, **110**:179-187.

Sauer, J.D. (1993). *Historical Geography of Crop Plants*: A Select Roster. CRC Press, Boca Raton, Florida, USA. 309 pp.

Schnathorst, W. C. (1981). *Life cycle and epidemiology of Verticillium*. **In**: Fungal Wilt Diseases of Plants. M. E. Mace, A. A. B. a. C. H. B. (Eds.), Academic Press, Inc., New York. pp. 81-111

Seelanan, T., Sch nabel, A. and Wendel, J.F. (1997). Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany.* **22**: 259-290.

Serdy, F. S., Berberich, S. and Sharota, E. (1995). Petition for determination of nonregulated status Bollgard® cotton lines 757 and 7076 (*Gossypium hirsutum*L.) with the gene from *Bacillus thuringiensis* subsp.kurstaki. Monsanto Company, St. Louis, Mo.

Sharon, E, Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O., Spiegel, Y. (2001). Biological Control of the Root-Knot Nematode Meloidogyne javanica by *Trichoderma*harzianum. *Phytopathol.***91**(7): 687-693.

Siddiquee, S., Yusuf, U., Hossain, K. and Jahan, S. (2009). In vitro studies on the potential Trichoderma harzianum for antagonistic properties against Ganoderma boninense. *Journal of Food, Agriculture & Environment*.**7**: 970 – 976.

Singh, A., Shahid, M., Srivastava, M., Pandey, S., Sharama, A. & Kumar, V. (2014). Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. *Virology and Mycology,***3**(1): 1–7.

Smith WC (1999) Production statistics. **In** WC Smith, JT Cothren, eds Cotton: Origin, History, Technology and Production. John Wiley & Sons, New York.

Smith, C.W. (1995). Cotton (*Gossypium hirsutum L.*). Pp. 287-349 in C.W. Smith, Crop Production: Evolution, History, and Technology. John Wiley & Sons, New York.

Smith, S. N. and Snyder, W. C. (1975). Persistence of *Fusarium oxysporum f. sp. vasinfectum*in fields in the absence of cotton. *Phytopathology,***65**:190-196

Smith, S. N., DeVay, J. E., Hsieh, W. and Lee, H. (2001). Soil-borne populations of *Fusarium oxysporum f. sp. vasinfectum*, a cotton wilt fungus in California fields. *Mycologia,***93**:737-743.

Snyder, W.C. and Hansen, H.N. (1940). The species concept in *Fusarium*. *American Journal of Botany* 27: 64-67.*Sydowia,* **54**: 9-22.

Summerbell, R. C., Krajden, S. and Kane, J. (1989). Potted plants in hospitals as reservoirs of pathogenic fungi. *Mycopathologia,* **106**: 13 – 22.

Susanto, A., Sudharto, P. and Purba, R. (2005). Enhancing biological control of basal stem rot disease (Ganoderma boninense) in oil palm plantations. *Mycopathol*. **159**:153–157.

Taghavi, S., vanderLelie, D., Hoffman, A., Zhang, Y.B., Walla, M.D., Vangronsveld, J., Newman, L., Monchy, S. (2010). *Genome sequence of the plant growth prmoting endophytic bacterium Enterobacter species* 638. PLoS Genet 6:e100943

Tesfaye Alemu and Kapoor, I. J. (2004). *In Vitro* evaluation of *Trichoderma* and *Gliocladiumspp* against Botrytis corm rot (Botrytis gladiolorum) of Gladiolus. *Pest Mgt .J. Ethiopia*, **8**: 97-103.

Thomson, N.J. (1966a). Cotton variety trials in the Ord valley, North Western Australia 4. Natural crossing of cotton. *Empire Cotton Growing Review,***43**: 18-21.

Tian, C.F., Zhou, Y.Z., Zhang, Y.M., Li, Q., Zhang, Y.Z., Li, D.F., Wang, S., Wang, J., Gilbert, L.B., Li, Y.R., Chen, W.X. (2012). Comperative genomics of rhizobia nodulating soybean suggests extensive recruitment of lineage specific gene in adaptation. *Proc Natl Acad Sci USA,***109**:8629–8634

Tisserant, E., Malbriel, M., Kuo, A., Kohler, A., Symeonidi, A., Balestrini, R., Charron, P., Duensing, N., Frey, N.F.D., Gianinazzi-Pearson, V., Gibert, L.B., Handa, Y., Herr, J.R., Hijri, M., Koul, R., Kawaguchi, M., Krajinski, F., Lammers, P.J., Masclauxm, F.G., Murat, C., Morin, E., Ndikumana, S., Pagni, M., Petipuerre, D., Requena, N., Rosikiewhicz, P., Riley, R., Saito, K., Clemente, H.S., Shapiro, H., Van Tuinen, D., Becard, G., Bonfante, P., Paszkowski, U., Shacha-Ahaill, A.Y.Y., Atuskan, G.A., Young, P.W., Sanders, I.R., Henrissat, B., Rensing, S.A., Grigoriev, M.N., Roux, C., Martin, F.

(2013). Genome of an arbuscular mycohrrizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci USA, **110**:20117–20122

Trikarunasawat, Ch. (2008). Biological control of rhizome rot of ginger during storage by antagonistic microorganisms and medicinal plant extracts. MSc Thesis, Kasetsart University, Bankok.

Vinale, F., Sivasithamparam, K., Ghesalberti, E., Marra, R., Lorito, M., Barbetti, M., Li, H., Woo, S., Lorito, M. (2008). A novel role for *Trichoderma* secondary metabolies in the interactions with plants. *Mol. Plant Pathol*. **72**: 80-86.

Vreeland, J.r., J.M. (1999). *The revival of colored cotton. Scientific American,***280**(4): 112-118.

Wakelyn, P.J., N.R. Bertoniere, A.D. French, D.P. Thibodeaux, B.A. Triplett, M.-A. Rousselle, W.R. Goynes, Jr. J.V. Edwards, L., Hunter, D.D. McAlister and G.R. Gamble. (2007b). *Cotton fibers.* Pp. 521-666 in M. Lewin, ed., *Handbook of Fiber Chemistry*, 3rd ed. CRC Press, Boca Raton, Florida, USA.

Wakelyn, P.J., N.R. Bertoniere., A.D. French., D.P. Thibodeaux., B.A. Triplett, M.-A., Rousselle., W.R. GoynesJ.r., J.V. Edwards., L. Hunter., D.D. McAlister and G.R. Gamble. (2007a). *Cotton Fiber Chemistry and Technology*. CRC Press, Boca Raton, Florida, USA. 176 pp.

Walter, S., Nicholson, P. and Dooha, F.M. (2009). Action and reaction of host and pathogen during Fusarium head blight disease. *New Phytologist,***185**: 54–66.

Watkins, G.M. (1981). *Compendium of cotton diseases*. APS Press, St. Paul MN, USA.

Weindling, R. (1932). *Trichoderma*lignorum as a parasite of other soil fungi. *Phytopathology*, **22**: 837–845.

Wendel, J.F. and R.C. Cronn. (2003). Polyploidy and the evolutionary history of cotton. *Advances in Agronomy,***78**: 139-186.

Wendel, J.F., Albert, V.A. (1992). Phylogenetics of the cotton genus (*Gossypium*): Character-State Weighted Parsimony Analysis of chloroplast DNA restriction sitedata and its systematic and biogeographic implications. *Systematic Botany,***17**: 115-143.

Whipps, J.M. (1987). Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *New Phytol*., **107**(1): 127-142.

Windels, C. E. (2000). Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in the Northern Great Plains. *Phytopathology,* **90**: 17-21

Wollenweber, H.W., and Reinking, O.A. (1935). *Die Fusarien ihre Beschreibung, Schadwirkung und Bekampfung. Berlin: Paul Parey.*

Yedidia, I., Srivastva, A., Kapulnik, Y., Chet, I. (2001). Effect of *Trichoderma*harzianum on microelement concentrations and increased growth of cucumber plants. *Plant Soil*, **235**:235-242.

Zehra, A., Dubey, M.K & Upadhyay. (2014). *Effect on salt, temperature and pH on the growth and sporulation of Trichoderma spp*. In: Souvenir of 4th International Science Congress, December 8th–9th, Udaipur, India, pp.70.

Appendix

Appendix 1 Descriptive Statistics (dual culture test)

Dependent Variable: PIRG

Trichoderma Isolates	Incubation periods	Mean	Std. Deviation	N
ChenT14	day 6	62.1622	4.68122	3
	Day 8	67.4157	1.94612	3
	Day 10	73.3945	1.58904	3
	Total	67.6575	5.54486	9
Control	day 6	.0000	.	1
	Day 8	.0000	.	1
	Day 10	.0000	.	1
	Total	.0000	.00000	3
GimT7	day 6	29.7297	6.19267	3
	Day 8	41.5730	5.14896	3
	Day 10	52.2936	4.20420	3
	Total	41.1988	10.77841	9
T131	day 6	68.9189	2.34061	3
	Day 8	74.1573	1.94612	3
	Day 10	78.8991	1.58904	3
	Total	73.9918	4.65175	9
T136	day 6	64.8649	4.68122	3
	Day 8	70.7865	3.89225	3
	Day 10	76.1468	3.17807	3
	Total	70.5994	5.97293	9
Total	day 6	52.0790	22.42059	13
	Day 8	58.5998	21.99803	13
	Day 10	64.7848	22.24507	13
	Total	58.4879	22.25856	39

Appendix 2 Multiple Comparisons (dual culture)

Dependent Variable: PIRG

	(I) Trichoderma Isolates	(J) Trichoderma Isolates	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	ChenT14	Control	67.6575*	2.50715	.000	60.2713	75.0436
		GimT7	26.4587*	1.77282	.000	21.2359	31.6815
		T131	-6.3343*	1.77282	.012	-11.5571	-1.1115
		T136	-2.9419	1.77282	.476	-8.1647	2.2809
	Control	ChenT14	-67.6575*	2.50715	.000	-75.0436	-60.2713
		GimT7	-41.1988*	2.50715	.000	-48.5849	-33.8126
		T131	-73.9918*	2.50715	.000	-81.3779	-66.6056
		T136	-70.5994*	2.50715	.000	-77.9855	-63.2132
	GimT7	ChenT14	-26.4587*	1.77282	.000	-31.6815	-21.2359
		Control	41.1988*	2.50715	.000	33.8126	48.5849
		T131	-32.7930*	1.77282	.000	-38.0158	-27.5702
		T136	-29.4006*	1.77282	.000	-34.6234	-24.1778
	T131	ChenT14	6.3343*	1.77282	.012	1.1115	11.5571
		Control	73.9918*	2.50715	.000	66.6056	81.3779
		GimT7	32.7930*	1.77282	.000	27.5702	38.0158
		T136	3.3924	1.77282	.337	-1.8304	8.6152
	T136	ChenT14	2.9419	1.77282	.476	-2.2809	8.1647
		Control	70.5994*	2.50715	.000	63.2132	77.9855
		GimT7	29.4006*	1.77282	.000	24.1778	34.6234
		T131	-3.3924	1.77282	.337	-8.6152	1.8304
LSD	ChenT14	Control	67.6575*	2.50715	.000	62.4830	72.8320
		GimT7	26.4587*	1.77282	.000	22.7998	30.1176
		T131	-6.3343*	1.77282	.002	-9.9932	-2.6754
		T136	-2.9419	1.77282	.110	-6.6009	.7170
	Control	ChenT14	-67.6575*	2.50715	.000	-72.8320	-62.4830
		GimT7	-41.1988*	2.50715	.000	-46.3733	-36.0243
		T131	-73.9918*	2.50715	.000	-79.1663	-68.8173
		T136	-70.5994*	2.50715	.000	-75.7739	-65.4249
	GimT7	ChenT14	-26.4587*	1.77282	.000	-30.1176	-22.7998
		Control	41.1988*	2.50715	.000	36.0243	46.3733

		T131	-32.7930*	1.77282	.000	-36.4519	-29.1341
		T136	-29.4006*	1.77282	.000	-33.0595	-25.7417
	T131	ChenT14	6.3343*	1.77282	.002	2.6754	9.9932
		Control	73.9918*	2.50715	.000	68.8173	79.1663
		GimT7	32.7930*	1.77282	.000	29.1341	36.4519
		T136	3.3924	1.77282	.068	-.2666	7.0513
	T136	ChenT14	2.9419	1.77282	.110	-.7170	6.6009
		Control	70.5994*	2.50715	.000	65.4249	75.7739
		GimT7	29.4006*	1.77282	.000	25.7417	33.0595
		T131	-3.3924	1.77282	.068	-7.0513	.2666

Based on observed means.
The error term is Mean Square(Error) = 14.143.
*. The mean difference is significant at the .05 level.

Appendix 3 Descriptive Statistics (volatile compounds)

Dependent Variable: PIRG

Incubation periods	Trichoderma Isolates	Mean	Std. Deviation	N
day 6	ChenT14	33.9623	7.12249	3
	Control	.0000	.	1
	GimT7	16.9811	18.84432	3
	T131	33.9623	18.19557	3
	T136	38.6792	17.05957	3
	Total	28.5196	17.69194	13
Day 8	ChenT14	41.8033	6.18839	3
	Control	.0000	.	1
	GimT7	27.0492	16.73818	3
	T131	42.6230	15.80926	3
	T136	43.4426	20.12792	3
	Total	35.7503	17.98472	13
Day 10	ChenT14	47.4074	5.59247	3
	Control	.0000	.	1
	GimT7	34.0741	15.12635	3
	T131	48.1481	14.28689	3
	T136	47.4074	20.64794	3
	Total	40.8547	18.26955	13
Total	ChenT14	41.0577	8.01766	9
	Control	.0000	.00000	3
	GimT7	26.0348	16.47364	9
	T131	41.5778	15.31759	9
	T136	43.1764	17.17407	9
	Total	35.0415	18.23933	39

Appendix 4 Pairwise Comparisons (volatile compounds)

Dependent Variable: PIRG

(I) Trichoderma Isolates	(J) Trichoderma Isolates	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
ChenT14	Control	41.058*	10.349	.006	9.072	73.043
	GimT7	15.023	7.318	.511	-7.594	37.640
	T131	-.520	7.318	1.000	-23.137	22.097
	T136	-2.119	7.318	1.000	-24.736	20.498
Control	ChenT14	-41.058*	10.349	.006	-73.043	-9.072
	GimT7	-26.035	10.349	.190	-58.020	5.950
	T131	-41.578*	10.349	.005	-73.563	-9.593
	T136	-43.176*	10.349	.003	-75.162	-11.191
GimT7	ChenT14	-15.023	7.318	.511	-37.640	7.594
	Control	26.035	10.349	.190	-5.950	58.020
	T131	-15.543	7.318	.442	-38.160	7.074
	T136	-17.142	7.318	.278	-39.759	5.475
T131	ChenT14	.520	7.318	1.000	-22.097	23.137
	Control	41.578*	10.349	.005	9.593	73.563
	GimT7	15.543	7.318	.442	-7.074	38.160
	T136	-1.599	7.318	1.000	-24.216	21.018
T136	ChenT14	2.119	7.318	1.000	-20.498	24.736
	Control	43.176*	10.349	.003	11.191	75.162
	GimT7	17.142	7.318	.278	-5.475	39.759
	T131	1.599	7.318	1.000	-21.018	24.216

Based on estimated marginal means

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

b. Adjustment for multiple comparisons: Bonferroni.

Appendix 5 Descriptive Statistics (Non volatile compounds)

Dependent Variable: PIRG

Incubation periods	Trichoderma Isolates	Mean	Std. Deviation	N
day 6	ChenT14	44.7761	13.67933	3
	Control	.0000	.	1
	GimT7	44.7761	11.26841	3
	T131	32.8358	4.47761	3
	T136	35.8209	11.26841	3
	Total	36.5098	15.01979	13
Day 8	ChenT14	37.3626	6.59341	3
	Control	.0000	.	1
	GimT7	34.0659	14.37000	3
	T131	43.9560	8.72226	3
	T136	48.3516	15.57961	3
	Total	37.7853	15.95955	13
Day 10	ChenT14	38.8430	7.57451	3
	Control	.0000	.	1
	GimT7	39.6694	18.93626	3
	T131	49.5868	3.78725	3
	T136	52.8926	13.11943	3
	Total	41.7673	17.18272	13
Total	ChenT14	40.3272	9.13984	9
	Control	.0000	.00000	3
	GimT7	39.5038	13.94766	9
	T131	42.1262	9.06159	9
	T136	45.6884	13.93163	9
	Total	38.6875	15.81314	39

Appendix 6 Multiple Comparisons (non volatile compounds)

Dependent Variable: PIRG

	(I) Trichoderma Isolates	(J) Trichoderma Isolates	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	ChenT14	Control	40.3272*	7.77536	.000	17.4208	63.2337
		GimT7	.8234	5.49801	1.000	-15.3739	17.0207
		T131	-1.7990	5.49801	.997	-17.9963	14.3983
		T136	-5.3611	5.49801	.864	-21.5584	10.8362
	Control	ChenT14	-40.3272*	7.77536	.000	-63.2337	-17.4208
		GimT7	-39.5038*	7.77536	.000	-62.4103	-16.5974
		T131	-42.1262*	7.77536	.000	-65.0326	-19.2198
		T136	-45.6884*	7.77536	.000	-68.5948	-22.7819
	GimT7	ChenT14	-.8234	5.49801	1.000	-17.0207	15.3739
		Control	39.5038*	7.77536	.000	16.5974	62.4103
		T131	-2.6224	5.49801	.989	-18.8197	13.5749
		T136	-6.1845	5.49801	.792	-22.3818	10.0127
	T131	ChenT14	1.7990	5.49801	.997	-14.3983	17.9963
		Control	42.1262*	7.77536	.000	19.2198	65.0326
		GimT7	2.6224	5.49801	.989	-13.5749	18.8197
		T136	-3.5622	5.49801	.965	-19.7594	12.6351
	T136	ChenT14	5.3611	5.49801	.864	-10.8362	21.5584
		Control	45.6884*	7.77536	.000	22.7819	68.5948
		GimT7	6.1845	5.49801	.792	-10.0127	22.3818
		T131	3.5622	5.49801	.965	-12.6351	19.7594
LSD	ChenT14	Control	40.3272*	7.77536	.000	24.2797	56.3748
		GimT7	.8234	5.49801	.882	-10.5239	12.1707

		T131	-1.7990	5.49801	.746	-13.1463	9.5484
		T136	-5.3611	5.49801	.339	-16.7085	5.9862
	Control	ChenT14	-40.3272*	7.77536	.000	-56.3748	-24.2797
		GimT7	-39.5038*	7.77536	.000	-55.5514	-23.4563
		T131	-42.1262*	7.77536	.000	-58.1738	-26.0787
		T136	-45.6884*	7.77536	.000	-61.7359	-29.6408
	GimT7	ChenT14	-.8234	5.49801	.882	-12.1707	10.5239
		Control	39.5038*	7.77536	.000	23.4563	55.5514
		T131	-2.6224	5.49801	.638	-13.9697	8.7249
		T136	-6.1845	5.49801	.272	-17.5319	5.1628
	T131	ChenT14	1.7990	5.49801	.746	-9.5484	13.1463
		Control	42.1262*	7.77536	.000	26.0787	58.1738
		GimT7	2.6224	5.49801	.638	-8.7249	13.9697
		T136	-3.5622	5.49801	.523	-14.9095	7.7852
	T136	ChenT14	5.3611	5.49801	.339	-5.9862	16.7085
		Control	45.6884*	7.77536	.000	29.6408	61.7359
		GimT7	6.1845	5.49801	.272	-5.1628	17.5319
		T131	3.5622	5.49801	.523	-7.7852	14.9095

Based on observed means.

The error term is Mean Square (Error) = 136.026.

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