Characterization of *Fusarium* species from hot pepper (*Capsicum anuum* L.) and In vitro antagonistic effect of *Trichoderma* species

M.Sc. THESIS

BY

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Characterization of *Fusarium* species from hot pepper (*Capsicum anuum* L.) and In vitro antagonistic effect of *Trichoderma* species

M.Sc. Thesis

By

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A Thesis

Submitted to the Department of Biology, School of Graduate Studies, College of Natural Sciences, Jimma University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Microbiology)

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MSc THESIS APPROVAL SHEET

APPROVAL SHEET

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I have incorporated the suggestion and modification given and got the approval of my advisors. Hence, I hereby kindly request the department to allow me to submit my thesis for external defense.

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We, the thesis advisors has evaluated the contents of the thesis and found it to be satisfactory, executed according to the approved proposal, written according to the standards and formats of the University and is ready to be submitted. Hence, we recommended the thesis to be submitted for external defense.

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Decision/suggestion of department graduate council (DGC) _____

Chairperson of DGC Name _____Signature _____Date_____

Chairperson of CGC Name _____Signature _____Date_____

DEDICATION

This Thesis is dedicated to my beloved father Megersa Kaba Bedanie, his effortful sacrifice, support and endless encouragement through my life to accomplish things in success!

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree in Applied Microbiology at Jimma University College of Natural Sciences and is deposited at the University Library to make it available to borrowers under the rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of an academic degree, diploma or certificate.

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Department: Biology

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ABBREVIATIONS AND ACRONYMS

AUDPC	Area under Disease Progress Curve
BARC	Bako Agricultural Research Center
CRBD	Completely randomized block design
CZA	Czapek's Dox agar
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
JUCNS	Jimma University College of Natural Science
MARC	Melkasa Agricultural Research Center
SNNPRS	South Nation Nationalities People Regional State

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Abstract

Hot pepper (Capsicum anuum L.) is the prominent type of Capsicum species grown in Ethiopia. It is important cash crop to Ethiopian smallholder farmers and an important agricultural commodity which contribute to export earnings. Fusarium wilt caused by Fusarium oxysporum f.sp.capsici is one of the major biotic factors which cause loss of pepper productivity up to 80%. The aim of this study was to isolate pathogenic Fusarium species from pepper plant, determine its pathogenicity and screening Ethiopia hot pepper varieties. Total 48 samples of Pepper plant materials were collected from the major pepper cultivating districts of Jimma Zone. Fusarium isolates characterized morphological and morphometrical, accordingly. Pathogenicity confirmed using Koch postulates. The efficacy of host resistance and biological control in the management of Fusarium wilt was investigated under greenhouse and in vitro dual culture assays, respectively. From the collected samples, based on culture characteristics 96 isolates were clustered in to 8 groups. Thereafter, the isolates were identified as Fusarium oxysporum and Fusarium species using morphological and morphometrical. Of these, Fo1 isolates showed high pathogenicity by causing disease on susceptible cultivar, the rest isolates were recorded weak to moderately pathogenic to the susceptible Mareko Fana variety. Isolate Fo1 was identified as the virulent isolate based on the computed result of disease severity index and Area under Disease Progress Curve. As a result, it was used to evaluate the level of disease resistance in 12 Ethiopian pepper varieties. Based on severity index 16% of Ethiopian pepper varieties were resistance to Fusarium isolate, 67% moderately susceptible and 17% susceptible. Melka Dera and Marko Fana Large pod were considered as promising varieties that showed highly resistance and resistance reaction, respectively. Regarding the efficacy of the biocontrol agents (Trichoderma isolate) on in vitro growth of Fusarium oxysporum and Fusarium species, significant variation has been observed. Trichoderma isolates were 100% effective on isolate Fo3. In conclude, Fusarium wilt damage of hot pepper can be managed using host resistance varieties and biological control, neverthetheless the efficacy and economic validity of these methods should be verified under multi location field studies.

Keywords: Disease Severity, *Fusarium* isolates, Pathogenicity, Resistance, Susceptible

1. Introduction

Pepper is vegetable crop belongs to genus *Capsicum* and family *solanaceae*. *Capsicum* species had been originated from Mexico in the North to Bolivia in the South of Latin America, where it has been part of human diet since about 7500BC. Most peppers commercially cultivated in the world belong to the species *C. annum* (Ali, 2006; Kumar *et al*., 2010; Russo, 2012). It grows well under warm and high humid conditions, but requires dry weather at maturity. *C. annum* gives the best green fruit yield and better seed set at 21^{o}C to 27^{o}C during the day and 15 to 20^{o}C at night. Pepper adapts well in sandy loam soil and well drained good clay loam (Lemma, 1998). Pepper production is found from the humid tropics, to the dry deserts, to the cool temperate climates. The ability of pepper to thrive under this range of climatic conditions has rendered it a common crop worldwide.

Pepper has important roles in various aspects of economy, food and pharmaceutics. It serves as row material for the processing industries since it serves as an important cash crop to farmers, and a source of employment to urban and rural populations. It has the highest content of vitamin C among all plants and has important medicinal properties such as prevention of heart disease, actuation of blood ambulation and antioxidant characteristics (Subha *et al*., 2017). The carotenoid pigments (capsanthin) are responsible for color change in pepper during ripening. Hot peppers are also excellent source of vitamin A, vitamin E, folic acid, calcium, potassium and other uses (i.e., flavonoids, capsaicinoids and carotenoids). It is also used for imparting taste and flavor, providing color to food, in preservative, pharmaceutical, perfumery and cosmetic products (Subha *et al*., 2017). Peppers are the world's second important vegetable ranking after tomatoes and it is the most produced type of spice utilized in flavoring and coloring foods.

The area cultivated with pepper worldwide is about 1,987,059 ha with a production of 36,092,631 tonnes for fresh pepper on about 1,856,641 ha with a production of 4,625,833 tonnes of dried pepper; a total area of 3,843,700 ha with a total production of 40.7 million tonnes with an average productivity of 10.59 tonnes per hectare (FAO, 2017). Asia is the largest producer, followed by Americas, Africa and Europe. India, China, Peru, Bangladesh and Pakistan are the major pepper producing countries in the world (FAO, 2017).

Ethiopia has good climatic and soil conditions for growing chillies. The most commonly grown type is the Mareko Fana variety, a pungent long chilli of dark to red appearance (pungency is at least twice as high as required for western food processors). Also grown are the smaller *Mitmita* chillies, an even hotter, red, small pepper (Herms, 2015). According to CSA (2015/2016), vegetables production accounted about 1.44% of the area under all crops at national level. However, of the total estimated area under vegetables, about 70.93% and 16.86% was under red peppers and Ethiopian cabbage, respectively (Lagiso, 2016). Pepper is the main part in the daily diet of most Ethiopian societies as local dishes (karia, berber). The average daily consumption of hot pepper by Ethiopian is estimated at 15g, which is higher than tomatoes and most other vegetables (MARC, 2004). Ethiopia is one of a few African countries that produce capsaicin and oleoresin for the export market from locally selected materials that contributed substantially to the national economy (Aklilu *et al*., 2018). The total area cultivated with hot pepper in Ethiopia is about 172,849 ha with a total production 370,744 tonnes with an average productivity of 2.14 tonnes per hectare (FAO, 2017).

Hot pepper production is, however, declining with time due to a number of biotic and abiotic stresses that have constrained production and productivity of this important crop. The main biotic factors are diseases due to fungi, bacteria and viruses, which have drastically restricted the yield potential and quality. Amongst these, *Fusarium* wilt has emerged as a serious problem in recent years (Ochoa and Ramirez, 2001; Egea et al. 2002). *Fusarium* wilt that is caused by *Fusarium oxysporum* is one of the most economically important diseases, and study on assessment of hot pepper diseases in South Nation, Nationalities and Peoples Region (SNNPR) of Ethiopia by Shiferaw and Alemayehu (2014) showed the occurrence of 30-55% *Fusarium* wilt incidence and confirmed *Fusarium* wilt as the leading fungal disease of pepper in the area. Total crop failure due to *Fusarium* wilt diseases has been common in the region and farmers are sometimes forced to leave their production due to excessive infection pressure in the field.

The disease management with chemicals is economically not viable and unsafe for the environment (Wani *et al*., 2014). To minimize these problems, researchers have sought to develop biological control agents for soil-borne plant pathogens that might be more

environments friendly. Regarding *Fusarium* wilt, effective means of control in general include the use of soil disinfestations and resistant/tolerant plant materials.

Most Ethiopian farmer assume when the seedling of the pepper wilted as shortage of water or because of high amount of sun light. However, many peppers cultivating area in Jimma Zone has face problems by wilt but the causal agents and the resistance pepper variety in the area have not been determined yet. Pepper is exposed to many pathogenic organisms wherever it is cultivated. Many soils borne fungal root rot and wilt pathogens such as *R. solani, M. phaseolina, F. oxysporum* and *F. solani* are reported to be widespread and attack pepper roots and stem causing severe losses in seed germination, plant growth and yield (Güney and Güldür, 2018). The identity, virulence level of *Fusarium* wilt causing pathogen and resistance pepper varieties in the pepper cultivating area of Jimma zone Ethiopia has not been well profiled.

Hence it is imperative to identify, characterize, evaluate its pathogenicity and identify *Fusarium* wilt resistant pepper variety that could be used as source of resistance for introducing this beneficial trait into commercially acceptable and high performing hot pepper cultivars in the area. Thus, keeping in view these research gaps, the present studies were undertaken with the following objectives:-

1.1. General objectives

The general objective of the study was to pathogenic *Fusarium species* from hot pepper and *In vitro* antagonistic effect of *Trichoderma* species

1.2. Specific objective

The specific objectives of the study were:-

- To isolate and identify *Fusarium* species from pepper field
- To determine the pathogenicity of *Fusarium* isolates to pepper variety
- To screen for pepper varieties resistant to pathogenic *Fusarium* isolates
- To test *in vitro* antagonism of *Trichoderma* isolates against *Fusarium* species

2. Literature review

2.1. Pepper origin classification and Distribution

The beginning of Capsicum species is expanded from Mexico in the North to Bolivia within the South of Latin America, where it has been portion of human count as diet since around 7500BC. They are cross-pollinated annual herb with diploid chromosome number. They are the source of capsaicin, the most commonly used spice in the world (universal spice). The genus Capsicum has five cultivated species *C. annuum var. annuum, C. frutescens, C. chinense, C. baccatum var. pendulum* and *C. pubescens*, which are differentiated, based on different morphological characters specially the color, shape, test and size of fruits, flowers, seeds and the pungency (Bose *et al.*, 2002). Archeological information, phytogeography and hereditary examinations have driven analysts to propose that *Capsicum annuum* was at first tamed in Mexico or northern Central America, *Capsicum frutescens* within the Caribbean, *C. baccatum* in swamp Bolivia, *Capsicum chinense* in northern swamp Amazonia, and *C. pubescensin* the mid-rise of southern Andes (Ali, 2006; Kumar *et al*., 2010;Russo, 2012).

The genus Capsicum has five cultivated species *C. annuum var. annuum, C. frutescens, C. chinense, C. baccatum* var. *pendulum* and *C. pubescens* which are differentiated based on different morphological characters specially the color, shape, test and size of fruits, flowers, seeds and the pungency (Bose *et al.*, 2002).

The fruit of capsicum has a variety of names, such as ‘chilli’, ‘chilli pepper’ or ‘pepper’ depending on place (i.e., differences between the English-speaking countries) and type of fruits. The term “chilli” in most of the world refers exclusively to the smaller, hot types of Capsicum (Concise Oxford Dictionary, 2010). *Capsicum* spp. contains approximately 20-27 species, 5 of which are domesticated: *C. annuum, C. baccatum, C. chinense, C. frutescens*, and *C. pubescens*, and are cultivated (Grown) in different parts of the world. Among the five species of cultivated *Capsicum*, *C. annuum* is one of the most common cultivated crops worldwide followed by *C. frutescens* (Bosland and Votava, 2003).

Spanish and Portuguese zest dealer spread pepper around the world. Pepper was presented to Spain in 1493, Britain in 1548 and Central Europe in 1585. From Europe, it went to Asia. Hot pepper maybe a warm-season vegetable but can be grown beneath a wide extend of temperatures

(15°C - 32°C) and dampness conditions. Right now the crop is cultivated in different nations around the world counting India, China, Pakistan, Indonesia, Sri Lanka, Thailand and Japan in Asia, and Nigeria, Uganda and Ethiopia in Africa (Bosland and Votava, 2003).

2.2. Nutritional content and other values of pepper

Pepper has numerous culinary preferences. It comprises various chemicals counting steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber and mineral components. Numerous pepper constituents are vital for nutritional value, flavor, smell, texture, and color. Peppers are low in sodium and cholesterol-free, wealthy in vitamins A and C, and are a great source of potassium, folic acid and vitamin E. Fresh green peppers contain more vitamin C than citrus natural products and fresh new ruddy pepper has more vitamin A than carrots (Marin *et al*., 2004). Two chemical groups delivered by pepper are capsaicinoids and carotenoids. The capsaicinoids are alkaloids that make hot pepper pungent. A large number of carotenoids give high dietary value and the color to pepper (Hornero-Méndez *et al*., 2002; Pérez-Gálvez *et al*., 2003).

As a condiment it has gotten to be basic in each Ethiopian home. It is grown for its pungent natural products which are utilized both as green and ready to give pungency and flavor to the food. Chilli other than giving pungency and ruddy color to the dishes is additionally a great source of vitamin (175 mg/100 g), vitamin A (870 mg/100 g) and vitamin B (0.59 mg/10 g). Besides from these, protein, fats, carbohydrates and traces of minerals are too found. The main case of pungency which was prior accepted to be a crystalline unstable alkaloid called capsaicin is presently found to be a blend of 20 associated components. The extricated capsaicin is utilized in torment emollients, beauty care products, and medications related to heart maladies. Pepper is additionally a wealthy source of ruddy pigmentes to be specific capsanthin, capsorubin, cryptoxanthin and related carotenoids which are esters of capsanthin. Oleoresin can too be gotten from peppers and is broadly utilized in western nations in nourishment preparation, beverage, and beauty care products businesses conjointly medication for the treatment of irritation. Pepper too invigorates spit and gastric juices and makes a difference in assimilation. It is utilized as a counter aggravation in thorny warm powders, skin treatments, beauty care products, and torment emollients. Pepper extricates are utilized in a wide run of solutions against tonsillitis, diphtheria,

the misfortune of craving, discontinuous fever, stiffness, sore throat, swellings and solidified tumors (AVRDC, 2003).

2.3. World production of pepper

The foremost important producers and exporters of pepper include India, Thailand, China, Ethiopia, Cote d'Ivoire, Pakistan, Bangladesh, Myanmar, Ghana and Viet Nam. India is a leading producer of dried pepper contributing near to 2,096,000 tonnes (45.3 %) of the world production taken after by Thailand 349,615 tonnes (7.5 %) and China 313,997 tonnes (6.7 %)(FAO, 2017). China, Mexico, Turkey, Indonesia, Spain, USA, Nigeria, Egypt, Algeria and Tunisia are top ten green peppers a leading producer of green/fresh pepper within the world. China contributing near to 17,795,349 tonnes of fresh pepper (49 %) of the world production taken after by Mexico 3,296,875 tonnes (9.1 %) and Turkey 2,608,172 tonnes (7.2%)(FAO, 2017). Pepper is considered to be one of the foremost important crops within the tropics. The area cultivated with pepper worldwide is about 1,987,059 ha with a production 36,092,631 tonnes for producing fresh pepper and around 1,856,641 ha with a production of 4,625,833 tonnes for producing dried pepper; a total area of 3,843,700 ha with a total production of 40.7 million tonnes with an average productivity of 10.59 tonnes per hectare (FAO, 2017).

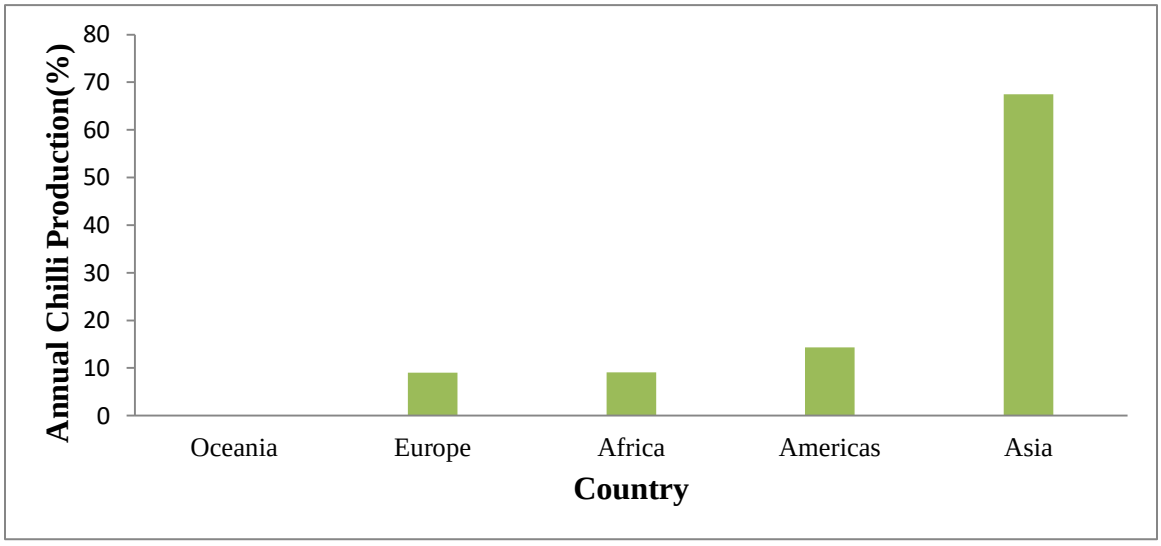

Figure 1: Production share of chillies (fresh peppers) by region (FAO, 2017)

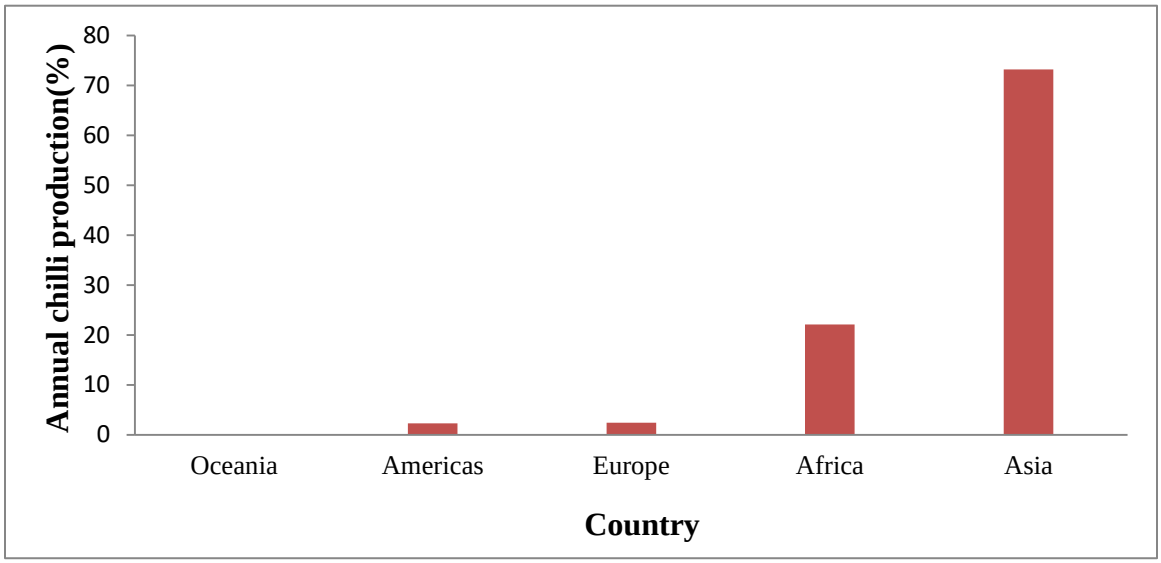

Figure 2: Production share of chillies (dry peppers) by region (FAO, 2017)

Pepper production in Ethiopia

In Ethiopia the total area under hot pepper production for green pod was to be about 10,000 ha with production of 64,041 tonnes. Hot pepper production for dry pod was to be about 162,849 ha with production of 306,703 tonnes. The total area cultivated with hot pepper in Ethiopia is about 172,849 ha with a total production 370,744 tonnes with an average productivity of 2.14 tonnes per hectare (FAO, 2017). In Ethiopia, the crop is cultivated at diverse ecological zones from sea level to 2000 m above sea level under rain fed and irrigated conditions (Lemma and Edward, 1994.). Yield is dependent on varieties and varieties themselves are considerably depending on a number of factors.

For most Ethiopian societies hot pepper is the main parts of the daily diet. The fine powdered pungent product is an indispensable flavoring and coloring ingredient in the common traditional sauce “Wot”, whereas the green pod is consumed as a vegetable with other food items. The average daily consumption of hot pepper by Ethiopian adult is estimated 15g, which is higher than tomatoes and most other vegetables (MARC, 2004).

2.4. Constraints of Pepper production In the World

Diseases caused by fungi, bacteria and viruses are the major imperatives to pepper production. Among the fungal diseases *Fusarium* wilt damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones. *Fusarium* wilt caused by *Fusarium* spp., Anthracnose disease caused by *Colletotrichum* species, bacterial wilt caused by *Pseudomonas solanacearum*, and mosaic disease caused by chilli veinal mottle virus (CVMV) or cucumber mosaic virus (CMV) are the foremost genuine dangerous destructive diseases of chilli (Poonpolgul and Kumphai, 2007). *Fusarium* wilt disease caused by *Fusarium* species is one of the most economically imperative diseases reducing marketable yield from 10% to 80% of the crop production in Ethiopia (Assefa *et al.*, 2015).

A number of biotic and abiotic stresses are a constraint in chilli production. The main biotic factors are maladies due to fungi, bacteria and viruses, which have drastically, limit the yield potential and quality (Ochoa and Ramirez, 2001; Egea *et al*. 2002). The major diseases affecting chilli production are anthracnose or ripe fruit rot (*Colletotrichum capsici*), Phytophthora leaf blight (*Phytophthora capsici*), *Fusarium* wilt (*Fusarium oxysporum* and *F. solani*), bacterial wilt (*Ralstonia salanacearum*), bacterial fruit rot (*Erwinia carotovora* and *E. atroseptica*), and poty- (ChVMV), tobamo- (PMMoV) and gemini- (TYLCV) viral diseases. Amongst these, *Fusarium* wilt has developed as a genuine issue in later a long time.

2.5. *Fusarium* wilt Ecology, Distribution, Host range and Economic importance

Fusarium spp. infects a wide range of hosts causing various diseases like vascular wilt, yellows, corm rot, root rot and damping-off (Naik, 2006). *Fusarium* wilt is a typical soil-borne disease and the fungus survives for several years in soil. The pathogen is extremely adaptable, variable and capable of long persistence in soil in the form of chlamydospores (Garret, 1960). *Fusarium* spp. produces different types of spores, i.e., macro-conidia, micro-conidia and chlamydospores (Nelson et al., 1981), which act as asexual spore and help in survival of the pathogen.

Pepper crop endures from numerous fungal, bacterial and viral diseases and among the fungal diseases, *Fusarium* wilt is one of the important disease due to the tremendous abdicate misfortunes. *Fusarium* wilt is a broad and noteworthy problem in pepper crop. It is economically harming the pepper industry and more harm is anticipated by appearance of virulent strains of

the pathogen. *Fusarium* species are known to cause diseases in many economically important host plants around the world including pepper, banana, cotton, legumes, maize, rice, wheat and others (Summerell *et al*., 2003). This pathogen too causes harm to crops belonging to other members of *Solanaceae* such as potato and tomato. Other important commercially grown plants affected by the *Fusarium* spp. include beans, carnation, chrysanthemum, peas and watermelon.

Fusarium wilt disease of pepper was first time reported by Leonian 1919 in New Mexico and named the pathogen as *Fusarium annuum*. Species of *Fusarium* such as *Fusarium oxysporum* and *F. solani* are the most common species found related with the diseased pepper crop in India and Pakistan (Naik, 2006; Joshi *et al.*, 2012). Bhuiyan (1989) also reported that *Fusarium oxysporum f.sp.capsici* was most overwhelming *Fusarium* species related with wilt in pepper. *Fusarium oxysporum* and *F. solani* was reported to cause incredible losses in pepper production in different parts of the world (Madhavi *et al*., 2006; Devika Rani *et al*., 2009). The seedling mortality due to *Fusarium* wilt incidence has been reported by Liang (1990) in China, which were 56 % and 36 % due to *Fusarium oxysporum* and *F. solani*, respectively. Naqvi (2004) reported 35 % wilt incidence in many states of USA and Assefa *et al*. (2015) reported 86.4 % wilt incidence in Ethiopia. *Fusarium* wilt is predominant in crops like tomato, eggplant and cucurbits with the estimated yield loss of 10-50% (Luckyanenko, 1991). *Fusarium* wilt is one of the important diseases in *solanaceous* vegetable crops such as tomato and pepper, and the disease is reported to cause 10-80 % yield loss around the world (Loganathan *et al*., 2013). In later a long time, the disease has risen as a serious problem in most of the irrigated pepper growing tracts of India, where the crop is grown particularly in black cotton soil leading to 25 % yield losses (Madhukar and Naik, 2004). The infection rate is recorded to differ from 2- 85% in different states of India where as of late in Kashmir valley the recorded wilt incidence changed from 4-40 % (Wani *et al*., 2014). In the same way, in case of wilt of tomato, the estimated yield losses in India changed from 30-40 percent (Anil and Rajkumar, 2013).

2.6. Disease Epidemiology

Environmental factors play an important role in deciding the severity and spread of any disease. The favorable host, pathogen and weather conditions lead to establishment of disease (Agrios, 2005). Thus, before proposing the management strategy of the disease, a thorough knowledge regarding the epidemiology of the disease should be studied. Spores are disseminated by the

wind, in ground water, or by movement of the contaminated soil, stake, or equipment. Environmental conditions like temperature, spore density and water potential influence the germination of *Fusarium* conidia (Stakheev *et al*., 2011). Spore production is triggered by the factors like nutrient sources, lights, metals, lipid signals and the chemistry of the plant host (Brodhagen and Keller, 2006).

The optimum growth of the genus *Fusarium* is found between 25 to 28°C, while the maximum growth is generally obtained at 28°C, inhibited above 33°C and not favored below 17°C (Cook and Baker, 1983). Generally, the dry weather condition and excessive soil moisture enhance the disease development. *Fusarium* spp. produce a wide variety of extracellular cell wall degrading enzymes (CWDEs), including pectin methyl esterases (PME), endo- and exo-polygalacturonases (PGs), xyllanases, cellulases, hemicellulases, proteases and pactate lyases, which disturb the structural barriers of the plant cell walls (Christakopoulos *et al.*, 1995; Garcia-Maceira *et al*., 2000). Mycelial growth and cell wall degrading enzymes and toxins produced by the pathogen may contribute to vascular plugging/occlusion, which lead to the development of a systemic vascular disease in host plants (Stover, 1970).

2.7. Pathogenic *Fusarium* species

The genus *Fusarium* was proposed by Link in 1809 for the species with fusiform, non septate spores borne on a mass of fungal tissue that has spore bearing, and this genus was afterward approved by Fries (1821). *Fusarium* species are not as it was common in tropical and temperate regions but these are too reported in cruel climatic conditions like desert, highland and arctic areas (Nelson *et al.*, 1994). Their far reaching dispersion may be due to their ability to colonize assorted biological specialties in most topographical regions of the world. The premise for all modern taxonomic systems in *Fusarium* is the work of Wollenweber and Reinking (1935) who given a sub-generic framework comprising of 16 sections, 65 species, 77 sub-specific varieties and forms. Snyder and Hansen (1941, 1945) diminished all fusaria to nine species in what has gotten to be known as the nine species system (Toussoun and Nelson, 1968). Since 1980s, the number of recognized species has expanded gradually and the current number of recognized species is more than eighty (Leslie and Summerell, 2006).

The genus *Fusarium* belongs to Kingdom Fungi, phylum Ascomycota, class Ascomycetes and order Hypocreales. *Fusarium oxysporum* is a highly ubiquitous, anamorphic species, while, *Fusarium solani* has Nectria haematococca and Haemanectria haematococca as teleomorphs. *F. solani* have both heterothallic and homothallic strains in nature, in any case, *F. oxysporum* have a potentially heterothallic origin (Irzykowska and Kosiada, 2011). The differential colors of different *Fusarium* spp. is observed due to presence of specific pigments (produced as by-products and involved in the enzymatic activities) such as rubrofusarin, aurofusarin, culmorin, javanicin, bostrycoidin, solanione and lycopersin. The size of *Fusarium oxysporum* genome has been estimated to range from 18.1 to 51.5 Mb (Migheli *et al*., 1993), including linear mitochondrial plasmids with chromosome numbers varying between7 to 14 (Ma *et al*., 2013). On the other hand, *F. solani* have 5-13 chromosomes and a total genome size of ~40 Mb (Suga *et al*., 2002). The inheritance pattern of *Fusarium* wilt resistance is governed by single dominant gene/monogenic dominant in nature (Manu *et al*., 2014).

Morphological characters are by far and away the most commonly used criteria for distingushing *Fusarium* species. Macro-conidia are the single most important cultural character in the identification of *Fusarium* species. In many cases the morphology of this spore alone is sufficient to identify a culture to species. The micro conidia themselves, the conidiogenous cell on which they are borne and the arrangement of the micro-conidia on and around the conidiogenous cell are important and potentially diagnostic characters. The most important diagnostic characters are the degree of curvature, relative length, general form and number of septations in macro- and micro-conidia (Leslie and Summerell, 2006). The micro-conidia are monophilidic and formed on relatively long false heads in *Fusarium solani*, though, on smaller false heads in case of *Fusarium oxysporum*.

Fusarium oxysporum have thin-walled, relatively slender, indistinctly septate macro-conidia, evenly curved fusoid with the widest part in the center (Booth, 1971). They are thin-walled, by and large 3-5 septate, fusoid-subulate and pointed at both the ends. It usually produces a pale to dark violet or dark magenta pigment in the agar but some isolates produce white mycelia. Be that as it may, *F. solani* have thick-walled, curved, dorsoventrally straight, relatively wider, stout(heavy) and robust(rough) macro-conidia in which a large percentage of the spores have the widest diameter in the upper half of the spore (Booth, 1971). Micro conidia of *Fusarium solani*

are somewhat wider, more oval in shape with thicker walls as compared to *Fusarium oxysporum*. It usually produces white to cream colored sparse mycelia and sporodochia often are produced in abundance and may be cream colored, blue or green on PDA. Chlamydospores may be formed singly, in pairs, in chains or in clumps which help in survival over winter in soil (Nelson et al., 1994).

2.8. Symptom of *Fusarium* wilt

Fusarium spp. infects a wide extend of hosts causing different diseases like vascular wilt, yellows, corm decay, root rot and damping-off. Symptoms are very variable but it includes combinations of vein clearing, downward curvature of leaves, wilting, chlorosis, necrosis and abscission. The vascular wilt symptoms indications show up at first as a slight yellowing and wilting of the upper leaves. The plants become permanently wilted within a couple of days and die. The dried foliage remains attached to the plant. The characteristic symptoms of the disease are brown vascular discoloration followed by upward and inward rolling of the upper leaves and subsequently wilting of the plants (Rivelli, 1989). The wilting symptoms appear as a result of severe water stress, mainly due to the vessel plugging/occlusion (Naik, 2006).

Fusarium wilt is a typical soil borne disease and the fungus survives for a few a long times in soil. The pathogen is extremely adaptable, variable and capable of long persistence in soil in the form of chlamydospores. *Fusarium* spp. produces different types of spores, i.e., macro-conidia, micro-conidia and chlamydospores (Nelson *et al*., 1981), which act as asexual spore and offer assistance in survival of the pathogen. Spores are dispersed by the wind, in ground water, or by movement of the contaminated soil, stake, or equipment. Environmental conditions like temperature, spore density and water potential influence the germination of *Fusarium* conidia (Stakheev *et al*., 2011). Spore production is triggered by the factors like nutrient sources, lights, metals, lipid signals and the chemistry of the plant host (Brodhagen and Keller, 2006).

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galacturonases (PGs), xyllanases, cellulases, hemicellulases, proteases and pactate lyases, which destroy the structural barriers of the plant cell walls (Garcia-Maceira *et al*., 2000). Mycelia growth and cell wall degrading enzymes and toxins secreted by the pathogen may contribute to vascular plugging/occlusion, which lead to the development of a systemic vascular disease in host plants (Stover, 1970).

2.9. Morphological and Cultural Variability of the Pathogen

The morphological and cultural features of the pathogen contribute to the distinguishing proof of pre-existing or any evolved pathogenic forms in any locality and these characteristics are also helpful in their taxonomic classification. Variation in cultural and morphological characteristics of *Fusarium oxysporum* and *Fusarium solani* causing wilt in pepper crop has been reported around the world by different workers (Loganathan *et al*., 2013; Ferniah *et al*., 2014). Different *Fusarium* spp. infecting Capsicum were isolated by the soil-plating methods given by Hassan et al. (1994), which given basis for easy and rapid isolation. According to Srobar (1978) found that a range of pH=0-6 was the most suitable for growth of *Fusarium* species, whereas a highly acidic medium was unsuitable for the sporulation of different *Fusarium* species. Daami-Remadi *et al.* (2006) observed that the temperature range from 25 to 30°C was optimum for extreme mycelia growth and sporulation of *Fusarium oxysporum* f.sp.*tuberose*, whereas, the temperature 30°C was most suitable for mycelia growth of *Fusarium solani.*

The cultural characteristics of *Fusarium* spp. were examined by Gautam *et al*., (2011) who reported Czapeck's Dox broth and Richard's broth medium as the most suitable for enormous growth of *Fusarium* spp., though, the optimum ranges of pH, temperature and incubation period were 4-6, 30-50°C and 6-7 days, respectively. As of late, Khilare and Ahmed (2012) have investigated the effect of different culture media, pH and temperature levels on mycelia growth of *Fusarium oxysporum* f.sp. *ciceris* and the best media were Czapek's dox agar & potato dextrose agar and the most suitable pH was 6.0-6.5, while, the maximum growth was found at 30°C. Rivelli (1989) has morphologically characterized *Fusarium oxysporum* infecting pepper and reported the macro conidia were septate and their size ranged from 41.0-46.8 x 4.5-4.7 µm. As of late, Bahar and Shahab (2012) shows high cultural and morphological variability among *Fusarium solani* isolates collected from different geographical regions of Iran and reported that the colonies were fluffy to fibrous based on mycelia texture; buff, umber, loteous, pale loteous,

ochreous and dark brown based on mycelia color; and long, medium and short macro conidial length.

Desai *et al*. (2003) studied the cultural variability among 15 isolates of *Fusarium oxysporum* f.sp. *ricini.* Among the isolates, six isolates produced moderate to growing in extreme abundance fluffy white mycelia, whereas nine isolates produced thin flat to slight fluffy pinkish mycelium on PDA medium. The number of spores ranged from 2.18 to 23.82 million per ml in potato dextrose broth (PDB) medium after 15 days of inoculation at 27 ± 2°C. The size of macro and micro conidia ranged from 17.5-70.0 x 3.5-5.25 µm and 5.25-14.0 x 3.5-7.0 µm, respectively. The colony diameter ranged from 62.67 to 73.67 mm after eight days of inoculation at 27 ± 2°C. Devika Rani *et al*. (2007) have reported variability in type of colony, growth margins, pigmentations, shape and sizes of micro-& macro-conidia, septations and sporulation among *Fusarium* isolates causing wilt in pepper. The size of macro and micro conidia ranged from 16.8-66.0 x 4.0-6.28 µm and 5.75-15.2 x 3.8-7.4 µm, respectively. The septations varied from 1-5 and the mycelia color varied from cream, pink to white.

Similarly, Joshi *et al*. (2013) identified and characterized the wilt pathogen in tomato, where thirty-nine isolates were identified as *Fusarium oxysporum* on the basis of macro-conidia characteristics that were thin walled generally 3-5 septate, fusoid, falcate macro-conidia with somewhat hooked apex and pedicillate base. The macro conidia of *Fusarium oxysporum* were in the range of 5.3-28.4 µm and the micro-conidia of 2.3-11.8 µm in size. Loganathan *et al.* (2013) studied on a large scale the cultural and morphological variability of *Fusarium* spp. infecting pepper and reported that *Fusarium solani* and *Fusarium oxysporum* were dominant species causing wilt in pepper, and the micro conidia were produced on large and small false heads in *Fusarium solani* and *Fusarim oxysporum*, respectively. The cultural and morphological variability among *Fusarium oxysporum* isolates in pepper were also observed by Ferniah *et al*. (2014) that showed white cottony aerial mycelia and purple on the reverse side with 4-5 cm diameter after 5 days of incubation on PDA medium. Macro-conidia were fusiform, with pedicellate basal cell, 27.0-46.0 x 3.0-4.5 µm in size and 3-5 septations. Micro-conidia were abundant, ellipsoid or fusiform without or with 1-2 septations, 5.0-15.0 x 2.2-3.5 µm in size. The cultural and morphological studies of *Fusarium oxysporum* isolates were investigated by Sultana *et al*. (2014) who showed that the fungus colony was cottony, thin flat to fluffy, thread like

spreading at periphery. The color of the colonies varied from creamy-white to white, whereas, some isolates produced pink violet colonies. Gupta *et al*. (2010) observed that the micro-conidia were abundant, one septate, oval to ellipsoidal in shape, hyaline in color and borne on short and plump monophialides, 3.31-10.21 x 2.01-3.51 μm in size. Macro-conidia were thin walled, delicate, 3-5 septate, cylindrical, straight to curved, sickle shaped, pointed at both ends and 22.69-30.76 x 3.42-4.24 μm in size.

2.10. Pathogenic variability of *Fusarium* Isolates

The pathogenic strains within *Fusarium oxysporum* have a high degree of host specificity. The host specificity of isolates of *Fusarium oxysporum* led Snyder and Hansen (1940) to sub-divide the species into formae speciales, based on the ability of the pathogen to infect a particular host or group of hosts (Armstrong and Armstrong, 1981).Although, virulence has been an extremely useful characteristic for differentiating isolates of *Fusarium oxysporum*. Moreover, it is influenced by a number of variables, including temperature, host age and method of inoculations (Hart and Endo, 1981). In order to find out the most efficient method of creating *Fusarium* wilt under controlled conditions for variability studies and for confirmation of resistance in the promising materials, three methods of inoculation through, soil inoculation, water culture technique and spore suspension method were evaluated by Mishra and Dhar (2005). The spore suspension method showed most effective with the highest wilting percentage followed by the soil inoculation but the results of water culture technique indicated an erratic pattern. However, the importance of water culture technique as a rapid test for testing pathogenicity and screening of selected genotypes against soil borne pathogen like *Fusarium* spp. was advocated by Haware & Nene (1994) and Nene & Kannaiyan (1982) in India.

Brasileiro *et al.* (2004) reported that *Fusarium solani* species complex (FSSC) were pathogenically and morphologically diverse, showing an extensive host range. El-Kazzaz *et al*. (2008) under greenhouse conditions found that *Fusarium solani* inoculated on pepper varieties. California Wonder were highly virulent, which showed typical wilt symptoms with 25.87 percent and 20.93 percent pre emergence and post emergence damping-off, respectively. Abdel Monaim and Ismail (2010) in Egypt isolated ten isolates of *Fusarium* spp. from pepper plants, which revealed that *Fusarium solani* isolate FP2 and *Fusarium oxysporum* isolate FP4 were highly pathogenic, while other isolates were moderate to less pathogenic to pepper varieties. Saengnak

et al. (2013) through pathogenicity test confirmed that *Fusarium oxysporum* isolates such as FoC1, FoC2, FoC3 and FoC4 isolates were pathogenic to pepper varieties. Chomthong 2, while the isolate FoC4 was highly virulent with highest disease index (DS=5.0). Ferniah *et al*. (2014) revealed that the *Fusarium oxysporum* isolate P1a was highly pathogenic, which caused wilting of pepper variety TM999 and Gantari with DSI scores of 0.4 and 0.63, respectively. Shafique *et al*. (2015) showed pathogenic variability in four strains of *Fusarium oxysporum* f.sp.*capsici* on ten different pepper cultivars and the results revealed that Strain-B was highly virulent with early symptoms and disease index of about 50% in pepper cultivar. Sky Red. Similarly, Nirmaladevi and Srinivas (2012) in case of wilt of tomato studied the pathogenic variability among the *Fusarium oxysporum* isolates on five susceptible varieties by root cut and dip inoculation methods. The isolates were categorized into four groups namely, highly pathogenic (18 isolates), moderately pathogenic (40 isolates), weakly pathogenic (20 isolates) and non-pathogenic based on the symptomatological variations in the test tomato varieties, whereas, non-inoculated tomato seedlings did not show any symptom.

2.11. Yield Loss in Ethiopia

Ethiopia is one of a few African countries that produce capsaicin and oleoresin for the export market from locally selected materials that contributed substantially to the national economy. In spite of tremendous potential use, good scope for processing, available export market as a spice powder and oleoresin and a wide range of available genetic resource, little effort has been made to improve quality and productivity of pepper, in the country (Aklilu, 2015). Among biotic factors that affect pepper production in Ethiopia, *Fusarium* wilt that is caused by *F. oxisporium* is one of the most economically important diseases, and it accounts for yield losses of up to 80% (Aklilu *et al.,* 2007). In recent years, the importance of the disease has been increasing and is given considerable attention by hot pepper producers and other stakeholders.

2.12. Disease Management of *Fusarium* wilt

There are constrained safe sources accessible against wilt pathogen in the germplasms of chilli throughout the world (Naik *et al.,* 2007). In this manner therefore, disease can be managed by cultural, biological and chemical means and by screening of germplasms/lines for resistance.

2.12.1. Cultural Methods

Utmost cultural management of *Fusarium* wilt is given emphasizes on avoiding or reducing the exposure of the seedlings to spores during transplanting by plowing tools. These includes avoiding transplanting, avoiding crop rotation, planting seeds with no detectable level of *Fusarium* spp., tillage operations that bury infected crop residues, burning of infected crop residues (Dill-Macky, 2008), mechanical chopping of infected residues to boost rate of decomposition and direct application of soil amendments (green manures, bentonite clay, urea, and spent lime) on residues can reduce the inoculum potential of residues (Dill-Macky, 2008). Flooding of fields and thus creating anaerobic conditions in the soil underneath in which apart from physical also chemical and microbial changes take place also applicable. All these were advised for *Fusarium* wilt management on hot pepper fields.

2.12.2. Chemical management

Chemical control is an important tool for managing different diseases including soil borne diseases. The over use of pesticides in past has led to several problems, such as environmental degradation, health hazards, pest resistance and decrease in population of beneficial insects (Groenewald, 2006).

Wilting in bell pepper (*C. annuum* L.) was investigated by Kelaniyangoda *et al.*, (2011) who reported *Fusarium* spp. was main causes of the wilting. The nematicide Metham as well as steam treated plants was highly effective against the pathogen with minimum wilt incidence. Madhavi and Bhattiprolu (2011) have reported that the integration of different treatments, including seedling root dip with carbendazim (0.1%), addition of vermicompost (100g/kg soil), drenching with fungicide carbendazim+mancozeb (0.2%) and soil application of *Trichoderma viride* (10g/pot) was highly effective against *Fusarium* wilt disease in chilli, which showed 89.8 percent reduction in the wilt incidence. Similarly, management of wilt complex disease in bell pepper (*C. annuum* L.) through integrated approach was reported by Rather *et al*. (2012). The seed treatment + seedling treatment + spraying with carbendazim+metalaxyl proved most effective, which showed 59.8 per cent reduction in wilt incidence under field conditions, as well as the integration of captan+metalaxyl with *Trichoderma harzianum* and *T. virens* was also found very effective.

2.12.3. Botanicals

Plants extracts and essential oils show antifungal activity against a large no of fungal diseases (Neela *et al*., 2014 and Javaid and Rauf, 2015). The plant extracts provide an effective measure for *Fusarium* wilt disease management and it represents an alternative to reliance on fungicides. The fungitoxic properties of different plant extracts against *Fusarium solani* have been investigated by Shivpuri *et al.* (1997). The results indicated that the ethanol extract of Allium sativum, Allium cepa, Withania somnifera, Lantana camara, Polyalthia longifolia, Tagetus erecta and Vinca rosea had fungitoxic effect against *Fusarium solani* and the maximum inhibition (80-85%) of the pathogen was observed with Allium sativum and Allium cepa. Similarly, Yelmame *et al*. (2010) worked out in vitro effects of different organic extracts against *Fusarium solani* infecting peppers. Least growth of the pathogen was recorded with extracts of neem (Large semi-evergreen tree of the East Indies) cake (59.8 %), followed by mustard cake (52.6 %), FYM (49.4 %), groundnut cake (44.8 %) and poultry manure (42.3 %). Singh and Kumar (2011) reported that the soil treatments with botanicals such as Mentha arvensis significantly reduced the *Fusarium* wilt of Chrysanthemum with the maximum (70.0%) disease control, followed by Tagetus patula (61.0%) and Daturastramonium (50.0%).

2.12.4. Antagonists

Different studies have shown beneficial interaction between plants and some fungal/endophytic bacteria, such as plant growth promotion and bio control potential against plant pathogens (Akrami and Yousefi, 2015; Srideepthi and Krishna, 2015; Sudarma *et al*., 2015). The effect of *Trichoderma* spp. on the incidence and intensity of *Fusarium* wilt and growth improvement in pepper were studied by Rini and Sulochana (2006). Amongst the antagonists applied, the least wilt intensity (10%) was recorded with application of *Trichoderma* isolate TR22 + Pseudomonas isolate P28 combination, which offered 69.9 percent disease control. Similarly, Sahi and Khalid (2007) reported that among the *Trichoderma* species, T. viride showed the best performance (62%), followed by *T. harzianum* (36%), *T. aureoviride* (24%), *T. koningii* (18%) and *T. pseudokoningii* (6%) in reducing the colony growth of *F. oxysporum* in sweet pepper (*Capsicum annuum*).Tariq *et al*. (2009) found that some strains of P. aeruginosa had nematicidal activity against Meloidogyne javanica, as well as strain PGPR-110 had inhibitory action against *Fusarium solani* and *Fusarium oxysporum*. The strain PGPR-110 was highly effective against

the pathogen, which also caused lysis of mycelium of *Fusarium solani* and *F. oxysporum* isolates.

Successful use of fungal bio control agents like *Trichoderma* spp. for the control of soil borne diseases caused by *Fusarium, Rhizoctonia, Sclerotium, Pythium* and *Phytophthora* in several crops have been reported by Cook and Baker in 1983. Later on, Singh and Zaidi (2002) reported that root dipping in antagonists (i.e., *Trichoderma* spp.) suspension (10^6cfu/ml) not only reduced the rate of disease severity but also enhanced the seedling growth in rice, tomato, pepper (chilli) and capsicum. Srideepthi and Krishna (2015) evaluated the efficacy of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *capsici* under in vitro conditions and concluded that *Trichoderma viride* as a potent bio fungicide to control *Fusarium* wilt in chilli. The results revealed that filtrate of *Trichoderma viride* exhibited 81.3 % of inhibition in potato dextrose agar well diffusion assay and 68.4 percent in dual culture assay, whereas, *T. harzianum* filtrate showed 54 and 34.2 % inhibition in well diffusion assay and in dual culture assay, respectively.

Bacterial Antagonists

The suppression of *Fusarium* wilt of chilli by chitinolytic bacteria was shown by Suryanto *et al.* (2010). The manifestation of bacterial (*i.e.,* BK08 isolate) suppression to *Fusarium* wilt was exhibited by increase in seedling height (ranged from 7.3-7.9 cm compared to 6.9 cm in control) and dry-weight (ranged from 2.7-4.3 mg compared to 2.3 mg in control). Oyetunji and Salami (2011) observed the effect of arbuscular mycorrhiza (AM) (*i.e.,Glomus mosseae*) and *Trichoderma koningii* as antagonists against *Fusarium* wilt of pepper. The result indicated that the inoculation with these antagonists effectively reduced the wilt pathogen and the efficacy was dependent on the sequence of inoculation of both the arbuscular mycorrhiza (AM) and the *T. koningii.* Similarly, Amaresan *et al*. (2014) investigated the antagonistic potential of endophytic bacteria against *Fusarium oxysporum* and the results revealed that most of the isolates showed antagonistic activity against *F. oxysporum*. The isolates BECS7, BECS4 and BECL5 showed growth promoting activity, reduction of disease incidence and high yield of chilli under field conditions.

2.12.5. Screening for Resistance Varieties

Host plant resistance has been a choice in all crop improvement programmers and it is perhaps the best method available to tackle soil borne diseases. Screening of genotypes/hybrids is an important aspect in resistant breeding program for the management of soil borne diseases worldwide especially against *Fusarium* wilt disease of economically important several crops (Purna, 2013; Kumar *et al.,* 2014; Shafique *et al*., 2015). For evaluation of chilli genotypes/hybrids against *Fusarium* wilt disease, different screening/inoculation technique and disease reaction scale have been put forth to identify the resistant genotypes/hybrids (Saha *et al*., 2007; Devika Rani *et al*., 2008). *Fusarium* wilt is a typical soil borne disease, which can be mitigated appropriately by the use of disease resistant cultivars. Further, resistant variety is essential not only in reducing losses due to disease but are also environment-friendly and less damaging to soil and water resources.

Kelaiya and Parakhia (2000) have determined the resistance of twenty chilli cultivars against *Fusarium solani* and reported that chilli cultivar GC-1 and GC-2 were resistant; Jwala, HC-9, HC-1, PBS-86-1 and PBS-86-2 were moderately resistant; whereas, HC-4, HC-10, HC-12, HC-5, HC-7, HC-13, HC-15, HC-11, HC-2, HC-3, HC-6, Reshampatto and Gholar were susceptible to highly susceptible. Ahmed *et al.* (1992) screened cultivars of hot pepper against *Fusarium* wilt under natural epiphytotic condition and reported the chilli cultivars Phule C-5, Musalwadi, SC-108, Arka Lohit, Pant C-2, SC-101, SC-137, LCA-248 and Jwahar-218 as resistant. Similarly, Nayeema *et al.* (1995) screened cultivars of hot pepper against *Fusarium pallidoroseum* causing wilt in chilli under artificial soil inoculations, sick soil and natural epiphytotic conditions. The results indicated that the lines Musalwadi, SC-120, Phule C-5, SC-335,SC-415, SC-107, SC-348, SC-108, LCA-304, Arka Lohit, Pusa Jwala, Pant C-2, SC-101, SC- 371, SC-137, SC-419, SC-451, SC-31, LCA-248, Jwahar-218 and SC-502 were showing resistance of various degrees varying from immune, highly resistant to moderately resistant in all the three screening procedures. Among the lines/cultivars, the cultivar Musalwadi was immune under all tested conditions.

Released varieties of pepper in Ethiopia

The plant requires a hot and dry climate free of frost and suitable agro ecological areas. Suitable altitude ranges for optimum production of pepper is between 1000 and 1800 m.a.s.l. According to MoANR (2016), the total hot pepper varieties released were 22 until 2016 (table 1).

Table 1: Ethiopian Hot Pepper Varieties (MoANR)

Varieties	Breeder	Year of release
Melka dera	MARC/EIAR	2016
Melka oli	MARC/EIAR	2016
Melka shote	MARC/EIAR	2006
Melka awaz	MARC/EIAR	2006
Oda haro	BARC/OARI	2005
Melka zala	MARC/EIAR	2004
MEX1TIZO RZ FI	Rijk Zwaan Zaadtlee en Zaadhandel.B.V/Joytech Plc	2016
Kume	BARC/OARI	2015
Dinsire	BARC/OARI	2015
Dame	BARC/OARI	2015
Vigro FI	MARKOS PLC	2015
Harbad FI	MARKOS PLC	2015
Serano	Mekamba PLC	2015
Sahem	Syngenta seeds B.V /Syngenta Agroservice AG Ethiopia	2013
Saidah	Syngenta seeds B.V./Syngenta Agroservice AG Ethiopia.	2013
CAPSI (SCH - 902 FI)	Vibha Seeds Ethiopia Private Limited Company	2012
SPICY (SCH -922 FI)	Vibha Seeds Ethiopia Private Limited Company	2012
SCH-925 FI	Vibha Seeds Ethiopia Private Limited Company	2012
SUPREME (SCH -942 FI)	Vibha Seeds Ethiopia Private Limited Company	2012
Serenade	Hazera genetics ltd (Greenline Trading PLC.)	2011
Melka Dima (Papri King)	MARC/EIAR	2004
Melka Eshet (P.Queen)	MARC/EIAR	2004

Where MARC=Melkasa Agricultural Research Center, BARC=Bako Agricultural Research Center

Other disease management

Soil solarization

Different approaches have been applied for the management of diseases caused by plant pathogens through sanitation, crop rotation, biological control, fungicidal application, breeding for disease resistance and soil disinfestations. The need for different methods of plant disease management required because none of them is perfect nor can anyone be used under all circumstances. The life cycles and survival of different pathogens may vary in different crop, thus requiring different management strategies. Therefore, any new method of disease management is improving our limited arsenal of control methods. This is particularly true with novel non chemical and eco-friendly approaches which are needed to replace hazardous chemicals (Scopa and Dumontet, 2007).

Soil solarization refers to disinfestations of soil by the heat generated from trapped solar energy (Katan, 1987). The basic principle of soil solarization is to elevate the temperature in a moist soil to a lethal level that directly affects the viability of certain organisms. The heating process also induces other environmental and biological changes in the soil that indirectly affect soilborne pests as well as survival of beneficial organisms (Katan, 1981). Soil solarization is an environment friendly method of using solar radiation for controlling soil borne plant pathogens by mulching the soil and covering it with tarp, usually with a transparent polyethylene cover for four weeks or more when irradiation and temperatures are high, to increase the maximal temperatures to a level lethal to pest. This effect is successful especially to control those plant pathogens and pests that are heat sensible and unable to survive at temperatures above37–40 °C.

Furthermore, soil pasteurization by solar heating gives positive effects against a broad number of seed weeds affecting crops cultivation, it is also very effective in managing the soil inhabitant insect pests, weeds, nematodes, plant pathogenic fungi and bacteria and also results in an increased growth response (IGR) of plants (Katan, 1981). Environmental factors such as soil temperature and soil moisture are known to have pronounced influence on the dynamics of soil respiration (Scopa and Dumontet, 2007). Use of this method has been reported to reduce the population of many soil borne pathogens including fungi bacteria and nematodes as well as weeds (Barbercheck and Broembsen; 1986; Verma *et al*; 2005).

Soil disinfection using heat and steam

Baker (1962) stated that 'most plant pathogenic micro-organisms, insects, viruses and weed seeds in soil may be destroyed at 140°F (±60°C) for 30 min'. Additional and detailed information about heat tolerance of soil micro-organisms, both pathogenic and non-pathogenic, became available in the next decades. Heavily infested moist soils were treated for 30 min at a temperature range thus establishing lethal temperatures. Thanks to these studies recommendation for soil sterilization became 70°C for at least half an hour to eliminate fungal and bacterial plant pathogens, parasitic nematodes and soil insects, slugs, worms and centipedes (Bollen, 1969, 1985).

Treatment of soil for 30 min at 60°C was sufficient to eliminate most plant pathogens and pests (Baker, 1962) enhanced the development of a less detrimental method than steam sterilization. Heating naturally infested soils in pot experiments at 60°C for 10 to 30 min eliminated Rhizoctonia solani and pathogens causing brown root rot e.g. Colletotrichum spp. and survival and growth of tomato and lettuce seedlings was better than at higher temperatures. This information led to the recommendation to disinfest soils at 65-75°C (Dawson et al., 1965).

Integrated disease management

The integrated management concept is one of the fundamental paradigms that have emerged in crop protection in the last 50 years and yet a matter for legislation as exemplified by the European Union that recently has establishes the integrated management as the fundamental procedure for the management of crop diseases, pests and weeds (Zadoxs, 2001). However, the integrated management is not a panacea for the control of plant diseases. It is an ecology-based approach aiming minimizing damage caused by diseases through 'the combined use of all available disease control measures, either simultaneously or in a sequence, through actions taken prior and after establishing the crop' (Rafael and Maria, 2011).

Diseases caused by soil-borne plant pathogens, if uncontrolled, are amongst the main limiting factors in crop production, particularly when availability of agricultural land and/or demand of food lead to intensive use and continuous cultivation. Plant pathogenic soil-borne fungi were differentiated by garret into two contrasting types, namely soil inhabitants and root inhabitants (Rafael and Maria, 2011). Regarded soil-inhabiting parasitic fungi as primitive, unspecialized

microorganisms infecting seedlings and juvenile root tissues, for which parasitism was incidental to an edaphic saprophytic existence and high competitive saprophytic ability. Conversely, root inhabitants were viewed as more highly specialized parasites that exhibit some host-specificity and a delayed destructive effect on the host plant. In the absence of their hosts, root-inhabiting fungi have a transitory existence in soil and low competitive saprophytic ability if any. A large majority of Fusaria are soil-borne. While those invading the plant root and foot cortex can be considered close to soil inhabitants, wilting Fusaria are a good example of root-inhabiting parasites (Rafael and Maria, 2011). Vascular wilts are caused by strains of the highly diverse *Fusarium oxysporum* species complex that display a high degree of pathogenic specificity to host species and cultivars. Those pathogenic strains are characterized by their ability to invade and colonize the vascular system of the host plant. These diseases are regarded amongst the most devastating and challenging of those that impair agricultural production worldwide (Rafael and Maria, 2011).

For diseases caused by soil-borne pathogens, such as *Fusarium* wilts, which are mainly monocyclic in nature, the control principles and methods should be targeted to excluding the pathogen, as well as reducing the amount and/or efficiency of the initial inoculum (Maloy, 1993). Therefore, integrated disease management strategies of those diseases within the framework of sustainable agriculture would include: (i) use of pathogen-free planting material; (ii) site selection to avoid planting into high risk soils; (iii) reduction or elimination of *Fusarium oxysporum* inoculum in soil; (iv) use of bio control agents for protection of healthy planting material from infection by resident or incoming inoculum subsequent to planting; (v) use of resistant cultivars regardless the level of resistance; and (vi) choice of cropping practices to avoid conditions favoring infection of the plant (Rafael and Maria, 2011).

3. Materials and methods

3.1. Description of the study areas and period

The study sample was collected from three selected districts of Jimma Zone, namely Kersa, Omo Nada and Sokoru(Fig.3), located at 22, 68 and 108 km, respectively, from Jimma town. Pepper is growing in six districts of Jimma Zone, such as Omo Nada, Kersa, Sokoru, Chora Botor, Tiro Afeta, Shebe Senbo and Seka Chekorsa. The first three districts were selected by considering the coverage of pepper production and diseases intensity. Characterization and pathogenicity test of *Fusarium* strains was carried out at College of Agriculture and Veterinary Medicine of Jimma University (JUCAVM).

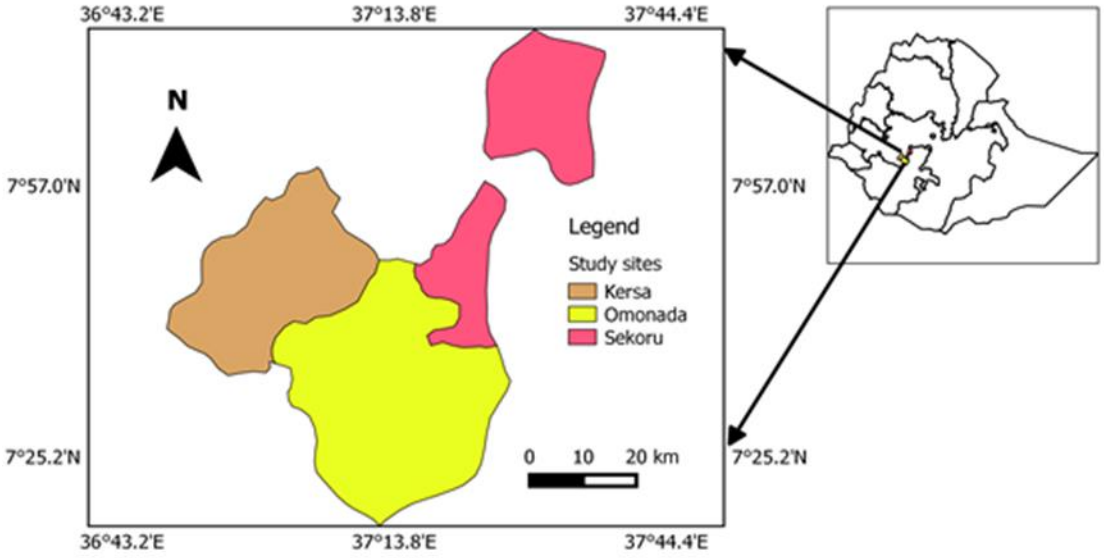

Figure 3: Sample collection sites

Kersa, Omo Nada and Sokoru are the districts of Jimma Zone, where the hot pepper plants are largely grown. The districts situated at the GPS coordinates of N07^{o}43.346' - N07^{o}47.596 and E037^{o}05.447'-E037^{o}20.830 with an altitude ranging from 1000 to 3340 meters above sea level (m.a.s.l). The area receives an average annual rainfall ranging from about 1,400 to 1,587 mm. The minimum and maximum daily temperatures of the area are 10^{o}C and 32^{o}C, respectively.

Kersa and Omo Nada are agro-ecologically characterized as highlands (10%), midlands (75%) and lowlands (15%). Moreover, 58.6% is arable, 17.3% pasture, 6.0% forest, and the remaining 18.9% is unusable, while in Sokoru the land comprises 36.6% arable, 16.8% pasture, 17.2%

forest and the remaining 29.4% is degraded. The major crops, vegetables and fruits grown in the districts are: - Maize, Sorghum, Teff, Bean, Pea, Coffee, Pepper, Potato, Sweet potato, Carrot, Cabbages, Avocado and Mangoes (Shumeta, 2012; Teshome, 2018; Yilma, 2018). The soil textural classes in selected districts dominated by clay soil with the pH range from 4.04-5.6 (Nigussie et al., 2013; Regassa, 2015; Deribe et al., 2018). The study was carried out from September 2018- August 2019.

3.2. Plant materials collection and preparation

A total of 48 plant samples were collected from pepper fields. Twenty four pepper samples from Omo Nada and 12 from each of Karsa and Sokoru districts were selected based on the coverage of pepper cultivation. Pepper fields were randomly selected at intervals of 3-5 km along the main and accessible rural roads. From each field, 5-10 pepper plants that showed a typical symptom (wilting and yellow color of leaves) were uprooted using soil probe at flowering stage by moving diagonally within selected fields. After removal of the adhered soil by gentle shaking, intact freshly infected roots and stems of pepper were transferred into sterile plastic polythene bag with proper labeling and transported to Plant Pathology Lab of JUCAVM and stored at 4^{o}C till used.

Pepper roots were cut into 1-2 cm pieces using sterile scissors. Root pieces were washed gently under tap water and surface sterilized with 70% ethanol for one minute, the root pieces were washed three times with sterile distilled water and placed on sterile filter paper to remove excess water (Güney and Güldür, 2018). Using sterile forceps, root pieces were transferred onto pre-solidified Malachite Green Agar (MGA) (g/l: 15 g Peptone, 1g KH_2PO_4, 0.5 g $MgSO_4 \bullet 7H_2O$, 2.5 mg Malachite green oxalate, 20 g Agar, 1 L distilled water) which was amended with 0.1 g/l chloramphenicol to exclude bacterial growth and inoculated plates were incubated at $27+2^{o}$C for 2-5 days (Castellá*et al*., 1997). Next, further purification of fungal colonies was performed by transferring the hyphal tip into fresh medium of MGA and incubated for 3-4 days at $27+2^{o}$C.

Purification of *Fusarium* isolates

Single spore culture technique was followed to obtain pure cultures of *Fusarium* spp. Fungal hyphae were flooded with sterile distil water from SNA (SpeziellerNährstof farmer Agar) (1 L of distilled water, 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \bullet 7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose, 20 g Agar) medium (Leslie and Summerell, 2006). Thereafter, using L-shaped glass

rod, fungal suspension was seeded on 3% water agar. Then, under dissecting microscope, a single conidium or hypha was re-inoculated into fresh SNA medium for further purification and characterization.

3.3. Morphological and morph metrical identification of *Fusarium* isolates

A thin of fungal hypha was taken from SNA medium using sterile inoculating loop, and transferred into a drop of lactone phenol blue (stain) on the slide and examined under 400X compound microscope (A.KRUSS Optronic GmbH, Hamburg). Morphological characterization (spore septation, shape, presence or absence of chlamydospore) and the morphometric measurements of macroconidia and microconidia (length and width in µm) were recorded from digital images using a camera fixed to microscope (3.1MP, APTINA Color CMoS, Germany) by pointing the cursor into the tip of the conidia image and drawing to the end of the conidia on the computer (which have software known as AmScope) connected with the microscope and the mean length and width of macro- and micro-conidia were calculated using Microsoft excel.

Moreover, the rate of radial growth of *Fusarium* isolates was measured using rulers within 24 h interval (starting from day 3) till the hyphae fully covered the Petridish that contained the medium of CzapekDox Agar(g/l;- 2g $NaNO_3$, 0.5 g KCl, 0.5 g $C_3H_7MgO_6P$, 0.01 g $FeSO_4$, 0.35g K_2SO_4, 30 g $C_{12}H_{22}O_{11}$, 12 g Agar) and Potato Dextrose Agar(g/l :- 20 g Potato infusion, 20g Dextrose, 20 g Agar). The species of *Fusarium* were tentatively identified using cultural characters, microscopic examination, morphometric parameters, and using standard manuals of Leslie and Summerell (2006).

3.4. Pathogenicity test

Marko Fana (susceptible cultivar) was used for this experiment. The seeds of this variety were obtained from Melkasa Agricultural Research Center (MARC). Reference strain of *Fusarim* sp. (*Fusarium oxysporum* f.sp.*capsici*) was also provided by MARC. The pathogenicity level of eight *Fusarium* isolates (Table 1), which were selected from 96 isolates were verified on 20 days old pepper seedlings of Marko Fana under greenhouse conditions. The concentration of the conidial suspension was adjusted to 4X 10^6 spore/mL using hemocytometer. The number of the spore can be adjusted by diluting the stock solution of 10-20days old cultures.

Twenty days old pepper seedlings were uprooted from seedling tray that grown under greenhouse conditions and the roots were washed under tap water to remove the soil. After trimming the root tips, the seedlings were dipped into 60 ml of *Fusarium* spore suspensions that contained (4×10^6 spore ml^{-1}) for 12 h including the reference strain (*Fusarium oxysporum* f.sp. *capsici*) (Herman and Perl-Treves, 2007).Then, inoculated seedlings (single plant per pots) were transplanted into 2 liter capacity pots that were filled with steam sterilized soil, compost and sand in the ratio of (1:0.5:0.5). All the treatments were arranged in a completely randomized design with three replications. A completely randomized design is a standard design for agricultural experiments where similar experimental units are grouped into blocks or replicates. Control plants were treated with 60 ml of sterilized distilled water. Inoculated and non-inoculated pepper seedlings were grown in greenhouse under a 12 h dark/12 h light photoperiod in the range of 14-30°C temperature. Data collections were carried out at 7, 14, 21, 28, 35, 42 and 49 days after inoculation (DAI).

Table 2: Formulation of treatments

No	Treatments	No	Treatments	No	Treatments
1	FI 1 + MF	11	FI 2 + MF	21	FI 3 + MF
2	FI 2 + MF	12	FI 3 + MF	22	FI 1 + MF
3	FI 3 + MF	13	FI 1 + MF	23	FI 2 + MF
4	FI 1 + MF	14	FI 2 + MF	24	FI 3 + MF
5	FI 2 + MF	15	FI 3 + MF	25	Fr1 + MF
6	FI 3 + MF	16	FI 1 + MF	26	Fr2 + MF
7	FI 1 + MF	17	FI 2 + MF	27	Fr3 + MF
8	FI 2 + MF	18	FI 3 + MF	28	MF c 1
9	FI 3 + MF	19	FI 1 + MF	29	MF c 2
10	FI 1 + MF	20	FI 2 + MF	30	MF c 3

Where: - FI= *Fusarium* isolate, MF= Marko Fana, Fr= Reference strain, MF c=Control plant

To determine the disease severity, a visual scale was designed based on the percentage of affected plants. Where 0= no symptoms; 1= initial symptoms or 1-10% chlorosis of leaves; 2= 10-20% chlorosis of leaves; 3= 20- 50% chlorosis of leaves; 4= > 50% chlorosis of leaves and initial symptoms flaccidity of the top leaves; 5= completely or the major part of the plant wilted or death (Ismail *et al*., 2010; Isaac *et al*., 2018). Disease severity index (DSI) was calculated following the formula of Ismail *et al*., (2010): $DSI\ (\%) = \Sigma\ \{(P \times Q)\} / (M \times N)] \times 100$, where P=severity score, Q=number of infected plants having the same score; M=Total number of plants

observed, N=Maximum rating scale number. Based on computed DSI (%), clearly differentiated *Fusarium* spps. isolates pathogenically in three different groups; pathogenic (DSI=41-100), moderately pathogenic (DSI=21-40) and weak (1-20) (Aklilu *et al*., 2018).

Moreover, pepper fresh and dry weight (after keeping in oven at 60^{o}C for 24-48 h), were recorded. The area under the disease progress curve (AUDPC) was calculated.

$$\text{AUDPC} = \sum_{i=1}^{n}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where: - n = total number of observations, y_i = initial injury intensity, t = day after inoculation, the unit for *y* in the sample data is %.

AUDPC values were also used to classify the level of pathogenicity among the different *Fusarium* strains (Isaac *et al.*, 2018).

3.5. Screening of Ethiopian pepper variety with *Fusarim* strains

Twelve Ethiopian hot pepper varieties were used for this experiments, in which six varieties:- Melka Awaz, Melka Dera, Mareko Fana, Melka Oli, Melka Shota and Melka Zala obtained from MARC, while Bako Local , Dame , Dinsire , Kume, Mareko Fana large pod and Oda Haro were obtained from Bako Agricultural Research Center(BARC) (Appendix Table 2). Based on the result of pathogenicity test, *Fusarium* isolate (FI1) was selected for screening of pepper varieties.

The seeds of all varieties were planted in seedling trays (i.e. contains 73 cells or small holes) that filled with steam-sterilized soil and sand (2:1: v: v) in a greenhouse. *Fusarium* spore suspension ($4X10^6$spore mL^{-1}) was prepared using hemocytometer. Trimmed root tips of three weeks old seedlings of each variety of pepper was dipped into 5 ml of *Fusarium* spore suspensions that contained ($4X10^6$ spore mL^{-1}) for 12 h (Herman and Perl-Treves, 2007).Then, inoculated seedlings (single plant per pots) was transplanted into 1 liter pots that were filled with steam sterilized soil, compost and sand in the ratio of (1:0.5:0.5). All the treatments were arranged in a completely randomized design with three replications. Control plants were treated with 5 ml of

sterilized distilled water. Inoculated and non-inoculated pepper seedlings were grown in greenhouse under a 12 h dark/12 h light photoperiod in the range of 14-30°C temperature. Data collections were carried out at 15, 30, 45, 60, 75, and 90 DAI.

Disease severity rating scale was rated based on a 5-point scale (Aklilu *et al*., 2018). Rating scale: 0 = no visible infection; 1=slight leaf yellowing; 2 = old lower leaf yellowing and plant wilting; 3=lower leaves shading and stunted plants; 4= all the leaves shedding and the stem collapsed /wilt; 5=Total plant death. The disease severity index (DSI) was calculated using the following formula: - DSI (%) = Σ {(P × Q)}/ (M × N)] × 100. Where P=severity score, Q=number of infected plants having the same score; M=Total number of plants observed, N=Maximum rating scale number. Based on computed DSI (%), each pepper variety categorized as: Highly resistant (HR) = 0%; Resistant (R) = <10%; Moderately Resistant (MR) =10-20%; Moderately susceptible (MS) =21-40%; Susceptible (S) =41-60%; highly susceptible (HS) >60%. Percent growth reduction of each variety due to pathogenic *Fusarium* species (FI1) was calculated in comparison to control.

3.6. In vitro antagonistic activity of *Trichoderma* isolates against *Fusarium* spp.

The potential bioagent *Trichoderma* spp. (T-GDb-2) was obtained from Jimma University Biology Department, Microbiology Research and Postgraduate Laboratory (isolated from tomato field by researcher Mat both for his MSc. thesis work) while pathogenic *Fusarium* species from study area were used for this experiment. Antagonistic activity of *Trichoderma* isolates against *Fusarium* spp. was evaluated using dual culture technique. Approximately, from 5 days old culture, 5mm mycelia disc was taken from tip of young hyphal growth both for antagonist as well as pathogenic fungi and placed on PDA at opposite side of each other (dual culture). Plates inoculated only with pathogenic fungus were used as control. Thereafter, the plates were arranged in randomized completed design with three replications and incubated at 28 ± 2°C for 7 days and within the interval of 24 h, the relative growth rates of mycelium was measured as a function of the incubation period or for 7 days starting from three days post incubation (DPI). The experiment was terminated when mycelial mats covered the medium surface in control treatment (Rai *et al*., 2016). All the plates were examined and percentage growth inhibition (PGI) of pathogenic fungi was calculated based on the method of Korsten and DeJager (1995).

$$\textbf{PGI (\%)} = \frac{\textbf{R-R}_1}{\textbf{R}} \textbf{ x 100}$$

Where:-

R=the distance in mm (radius) from the point of inoculation to the colony margin in control

R_1=the distance of fungal growth from the point of inoculation to the colony margin in treated plate in the direction of the antagonist (dual culture).

The effectiveness of *Trichoderma* isolates was determined using the following inhibitory scale (Sangoyomi, 2004):-

≤ 0% inhibition (not effective),

>0-20% inhibition (slightly effective)

>20-50% inhibition (moderately effective),

>50-<100% inhibition (effective)

100% inhibition (highly effective)

Data analysis

Rate of mycelia radial growth, mean size or length X width of micro and macro-conidia and percentage of diseases severity index were calculated using Microsoft office excel. Analysis the variance (ANOVA) of the AUDPC, plant length, weight and percentage growth inhibitions were performed with the SAS version 9.3 (2012). The mean values of the treatments were compared using the Tukey test ($p \leq 0.05$).

4. Results

4.1. Isolation and identification of *Fusarium* spp. from pepper plant

Cultural characteristics

From 48 samples collected from three districts, a total of 96 *Fusarium* isolates were obtained. Based on cultural characteristics and mycelial growth, the isolates were clustered into eight distinct of *Fusarium* isolates, such as FI1, FI2, FI3, FI4, FI5, FI6, FI7 and FI8. Cottony mycelia with white to pink pigmentation and circular shape of fungal colony were predominantly observed on PDA medium. Isolates belong to FI1, FI2, FI3, FI4, FI5 and FI8 was showed rapidly growth whereas FI6 and FI7was observed as slow grower (Table 2 and Fig. 4).

Table 3: A five days old cultural characteristics of *Fusarium* isolates on PDA medium.

	Colony morphology					
Isolates	**Growth rate**	**Shape**	**Front view**	**Back view**	**Arial growth**	**Mycelium**
FI1	Rapid	Circular	White purple	Brown	Fluffy	
FI2	Rapid	Circular	White purple	Grey	Fluffy	
FI3	Rapid	Circular	White	Brown	Fluffy	
FI4	Rapid	Circular	White	White	Fluffy	
FI5	Rapid	Circular	White	White	Cottony	
FI6	Slow	Circular	White	White	Flat	
FI7	Slow	Circular	White	Pale yellow	Flat	
FI8	Rapid	Circular	White purple	Pink	Fluffy	

Where FI=*Fusarium* isolates; PDA=Potato Dextrose agar

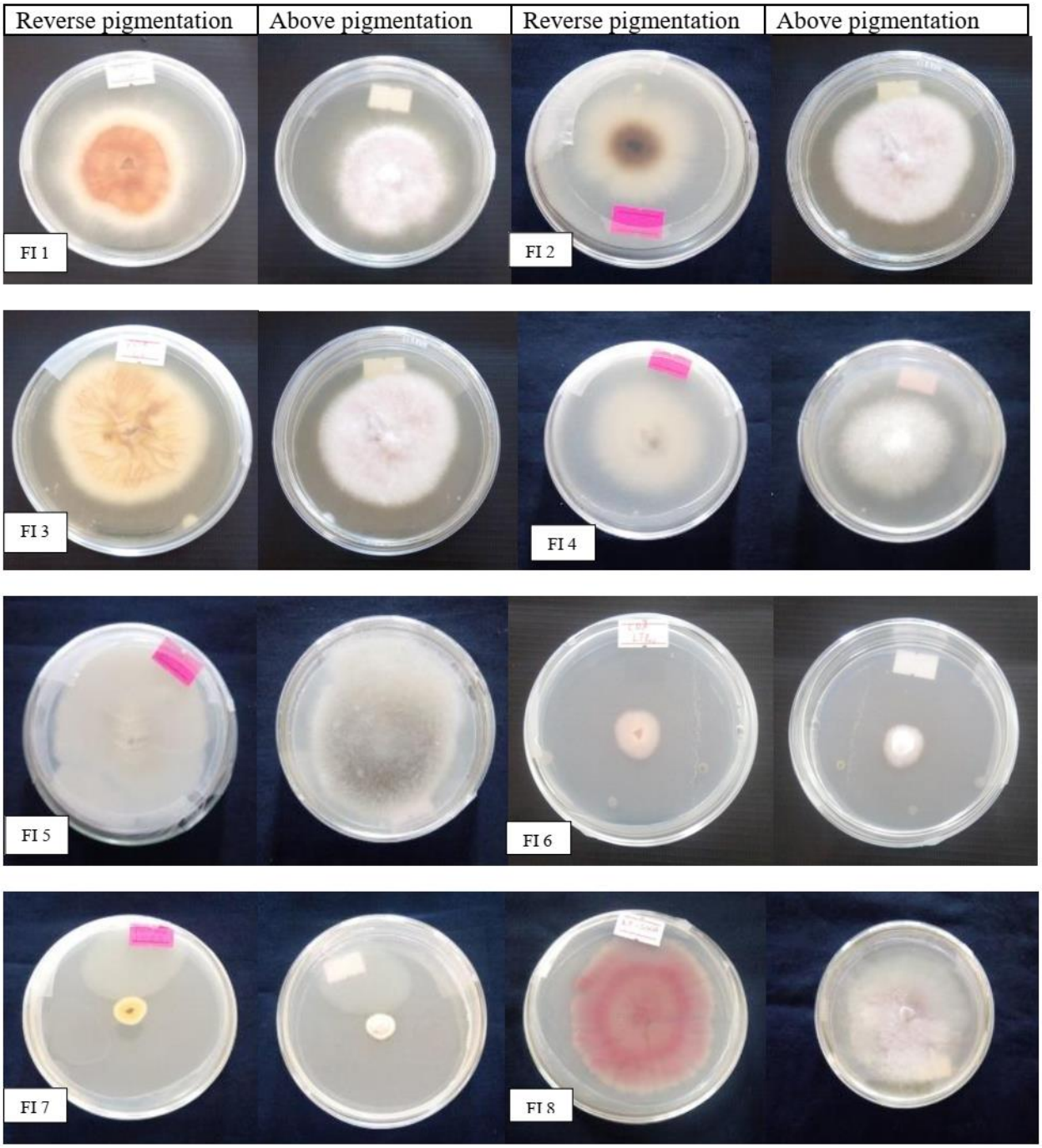

Figure 4: Cultural characteristics of the isolates

Radial Growth of the Isolate

In both of fungal growth medium (PDA and CZA), all of the *Fusarium* isolates showed slight variation in rate of mycelia radial growth. Similar to reference strain, FI1 and FI8 were formed the highest radial diameter (90 mm) on CZA, while 86 mm (FI2) and 82 mm (FI1) radial

diameter recorded on PDA medium (Table 4). CZA was enhanced the growth of *Fusarium* isolates.

Table 4: Rate of mycelia radial growth of *Fusarium* isolates on PDA and CZA media

Isolates	Radial diameter (in mm) from 3-7 days									
	PDA					CZA				
	3	4	5	6	7	3	4	5	6	7
FI1	38	54	60	65	82	40	55	70	82	90
FI2	38	55	66	75	86	40	52	65	80	85
FI3	30	46	55	63	72	17	22	33	43	50
FI4	35	50	60	70	80	36	50	60	76	82
FI5	46	55	60	65	68	38	50	62	74	81
FI6	30	36	41	45	50	20	30	34	38	40
FI7	20	27	31	35	40	17	23	27	31	35
FI8	38	56	65	73	78	43	56	64	85	90
Ref	38	50	58	65	85	40	58	70	83	90

Where: - PDA; Potato dextrose agar, CZA; Czapek's Dox agar, Ref; Reference strain.

Morphometric of conidia and Chlamydospores

Moreover, during microscopic examination, the macro conidia with fusoid (narrow from the tip and wider part in the middle), relatively slender and septation ranged to 1-5 was observed. The micro conidia of all isolates were abundant, had no septate, oval to ellipsoidal in shape. All of the *Fusarium* isolates were produced chlamydospores (Table 5 and Fig. 5).

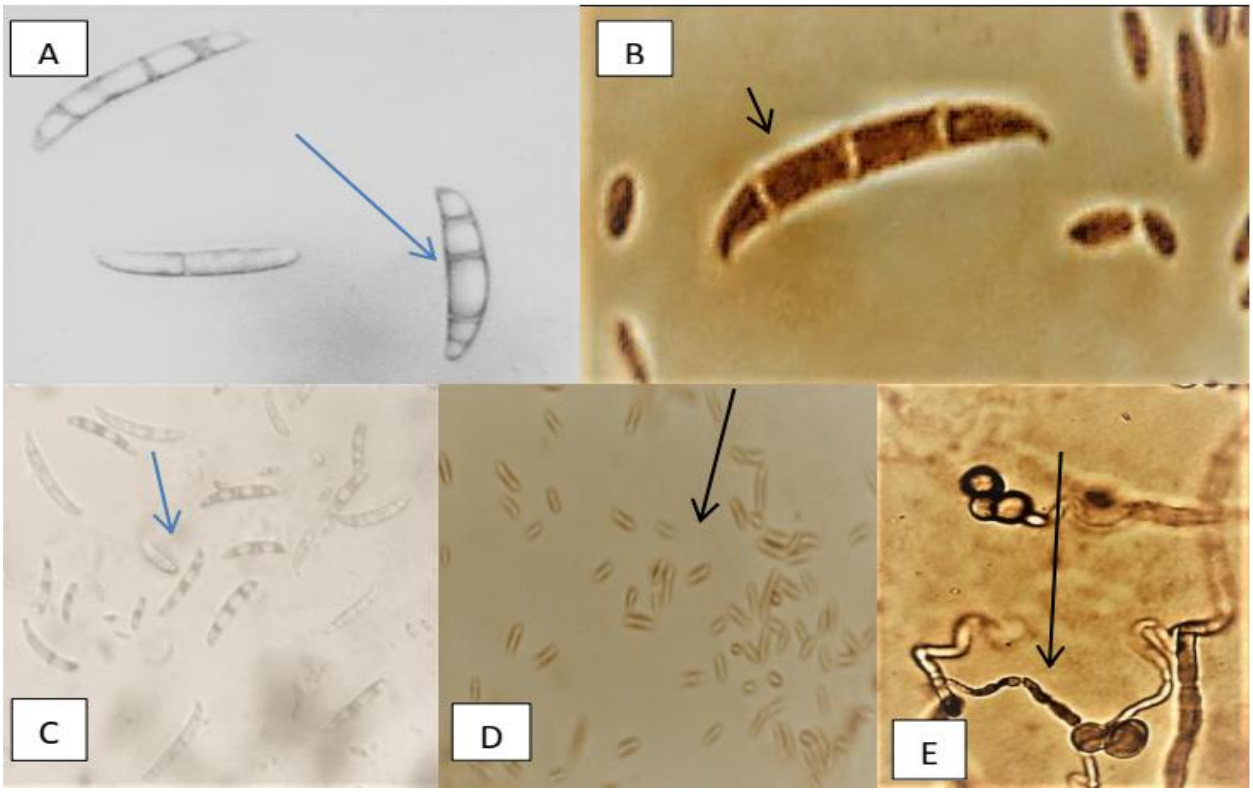

Figure 5: Microscopic features (400X) of *Fusarium* species isolated from diseased hot pepper plants in Jimma zone Ethiopia. A: Macroconidia Fusoid. B: Macroconidia Slender. C: 1-5 septation. D: Microconidia. E: Chelamadospore

Table 5: Conidial septation and chlamydospores on *Fusarium* isolates

Isolates	Conidial septation	Chlamydospores
FI1	1, 3, 4 and 5	smooth/rough, single/chain, terminal/intercalary
FI2	1, 3, 4 and 5	smooth/rough, single/chain, terminal/intercalary
FI3	1, 3 and 5	smooth/rough, single/chain, terminal/intercalary
FI4	1, 2 and 3	smooth, single, terminal
FI5	1, 2 and 3	smooth/rough, single/chain, terminal/intercalary
FI6	1, 2 and 3	smooth, single, terminal
FI7	1, 2 and 3	smooth, single, terminal
FI8	1, 3 and 5	smooth/rough, single/chain, terminal/intercalary

Size of conidia

Various sizes of micro and macro-conidia were observed among isolates. The mean size or length X width (µm) of micro and macro-conidia are ranging from 5.12 x 1.32 - 10.12 x 1.53, and 19.80 x 1.23 - 42.06 x 1.48 µm, respectively. The biggest mean size of micro conidia was recorded in FI1 (10.12 x 1.53 µm), while macro-conidia in FI2 (42.06 x 1.48 µm). On the other

hand, the smallest mean size of micro conidia obtained from FI6 (5.12 x 1.32 µm), whereas macro-conidia of FI7 (19.80 x 1.23 µm) (Fig. 5 and Table 5).

Table 6: Morphometric of conidia in *Fusarium* spp. isolated from the districts of Jimma Zone

Isolates	Micro-conidia dimensions		Macro-conidia dimensions	
	Range	**Mean size***	**Range**	**Mean size**
	Length X Width(µm)	Length X Width (µm)	Length X Width (µm)	Length X Width (µm)
FI1	8.03-12.50 x 1.06-1.45	10.12 x 1.53	18.27-71.78 x 1.31-2.54	40.31 x 1.24
FI2	7.04-10.00 x 1.22-1.64	9.02 x 1.59	15.25-72.34 x 1.27-2.77	42.06 x 1.48
FI3	6.84-9.24 x 1.20-1.40	8.08 x 1.31	11.00-58.85 x 1.40-1.85	35.95 x 1.27
FI4	7.00-9.00 x 1.12-1.20	7.23 x 1.14	15.52-53.37 x 1.28-2.25	28.19 x 1.54
FI5	6.00-9.81 x 1.47-2.40	8.41 x 1.44	15.00-45.00 x 1.50-3.00	35.69 x 1.93
FI6	5.00-6.40 x 1.00-1.38	5.12 x 1.32	13.25-26.00 x 1.0-1.2	26.01 x 1.12
FI7	4.43-6.30 x 0.90-1.30	5.85 x 1.21	10.00-24.03 x 1.21-1.27	19.80 x 1.23
FI8	6.00-9.09 x 1.00-1.94	6.42 x 1.64	16.00-36.88 x 1.20-1.54	31.17 x 1.37

*****mean of 20 observations; FI=*Fusarium* isolates

Identification of *Fusarium* species

Based on aforementioned characteristics, such as rate of mycelia radial growth, chlamydospores and morphometric of conidia, shape, pathogenicity test(later), and using keys and description of the *Fusarium* Lab Manual (Leslie and Summerell, 2006), six isolates were identify as *Fusarium oxysporum*, while FI6 and FI7 designated as *Fusarium* species.

4.2.Pathogenicity test

The pathogenicity was proved on susceptible pepper cultivar on green house by root dip inoculation method in which the spore suspensions of individual isolates of *Fusarium oxysporum* were inoculated. The pathogenicity of the *Fusarium* spp. isolates were ascertained on the basis of the ability of each of the isolates to cause disease and showing temporal variation in appearance of the specific symptoms such as yellowing, wilting or plant death (leaf yellowing, leaf yellowing + plant wilting or, leaf yellowing + plant wilting + plant death) in pepper (Fig.6). The appearance of the specific symptom/disease severity varied depending on the pathogenicity or virulence level of specific isolate of *Fusarium oxysporum*. The plants inoculated with the specific isolate, showed typical symptoms starting from first to sixth week of inoculation in which the leaves presented yellowing, flaccidity curling of top leaves and wilting. Necrosis and a

brownish discoloration were also observed on roots and stem as shown in Fig. 6. Furthermore, the inoculated fungi were re-isolated from plants that showed witling symptoms, while no symptoms were found on control plants, for this reason the Koch's postulates were confirmed.

Pathogenic variation

The results of pathogenicity test showed, there were differences among isolates in terms of their ability to cause disease. Based on the results AUDPC, two isolates were identified as highly pathogenic, one moderate, and the rest observed as weak. The less susceptible of Marko Fana to five isolate indicated that, the pathogenicity is strain specific. Symptoms were observed 14-21 days after inoculation. The symptoms were begun with yellowing leaves, shading, flaccidity and wilting. Moreover brownish discoloration was observed on roots and vascular tissues (Table 7 and Fig. 6).

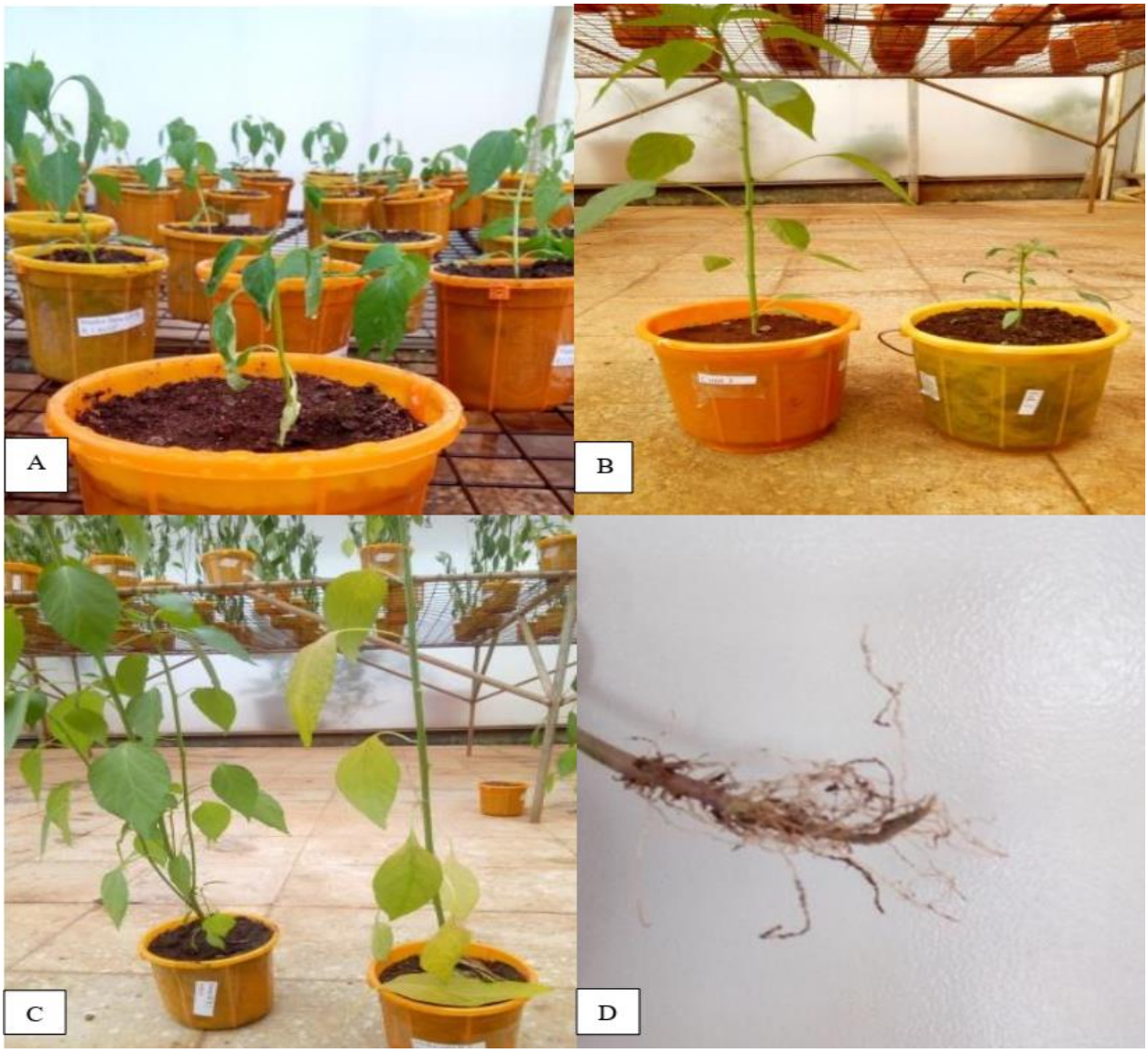

Figure 6: Pathogenicity of *Fusarium* isolates to the pepper variety of Marko Fana (A) wilting and curling symptom, (B) control v inoculated stunted growth, (C) yellowing and shedding and (D) infected root discoloration

The fresh weight of Marko Fana was various with the isolates of *Fusarium*. Compared to control, fresh weight of Marko Fana was reduced by 82.5 and 81% and dry weight by 88.9 and 86.9 % due to FI5 and FI1 isolates, respectively. Based on disease severity index, *Fusarium oxysporum* isolate (Fo1) was pathogenic as the symptom appeared very early in most severe form (Table 6).

Table 7: Pathogenicity test of *Fusarium* spp. to Mareko Fana, the susceptible pepper variety.

***Fusarium* isolates**	**AUDPC**	**DSI (%)**	**Severity/pathogenicity***	**Fresh weight (g)**	**Dry weight(g)**
Fo1	1330.5[a]	41.66	Pathogenic	3.16	0.53
Fo2	362.13[c]	31.66	Moderately pathogenic	8.26	1.54
Fo3	223.42[c]	23.33	Moderately pathogenic	12.7	2.85
Fo4	892.50[ab]	36.66	Moderately pathogenic	6.4	1.49
Fo5	1114.50[a]	38.33	Moderately pathogenic	2.9	0.45
FI6	157.50[c]	28.33	Moderately pathogenic	13.5	2.88
FI7	213.50[c]	15.00	Weak	14.33	3.47
Fo8	395.50b[c]	20.00	Weak	11.6	2.38
Control	-		-	16.6	4.07
LSD	0.51				

* Pathogenic: DSI= 41-100%, moderately pathogenic: DSI=21-40%, weak: DSI=1-20%

4.3. Reaction of hot pepper varieties to *Fusarium* wilts diseases

Except Melka Dera, all pepper varieties (11) were developed diseases symptom with different level of severity. Based on severity index rating, the varieties were grouped into 4 resistance level namely; highly resistant (0%), resistant (<10%), susceptible (41-60%) and moderately susceptible (21-40%). Melka Dera recorded as highly resistant (DSI=0) variety, while Melka Zala and Mareko Fana were identified as susceptible (DSI= 41-60%) (Table 8)

Table 8: Diseases severity index along with resistance levels of Ethiopia hot pepper varieties to *Fusarium* isolates (Fo1)

S.No.	**Pepper Varieties**	**Disease severity Index (%)**	**Level of resistance**	**Seed source**
1	Mareko Fana	41.1	Susceptible	MARC
2	Melka Oli	21.1	Moderately susceptible	“ “
3	Melka Zala	43.3	Susceptible	“ “
4	Melka Awaz	35.5	Moderately susceptible	“ “
5	Melka Shota	22.2	Moderately susceptible	“ “
6	Melka Dera	0	Highly resistant	“ “
7	Oda Haro	26.7	Moderately susceptible	BARC
8	Bako Local	26.7	Moderately susceptible	“ “
9	Dinsire	36.6	Moderately susceptible	“ “
10	Dame	27.7	Moderately susceptible	“ “
11	Kume	24.4	Moderately susceptible	“ “
12	Mareko Fana large pod	8.8	Resistant	“ “

Where: - MARC; Melkassa Agricultural Research Center, BARC; Bako Agricultural Research Center

Out of 12 pepper varieties, 67% of them were moderately susceptible to *Fusarium* wilt diseases; however, highly susceptible varieties were not recorded during greenhouse experiments (Fig. 7).

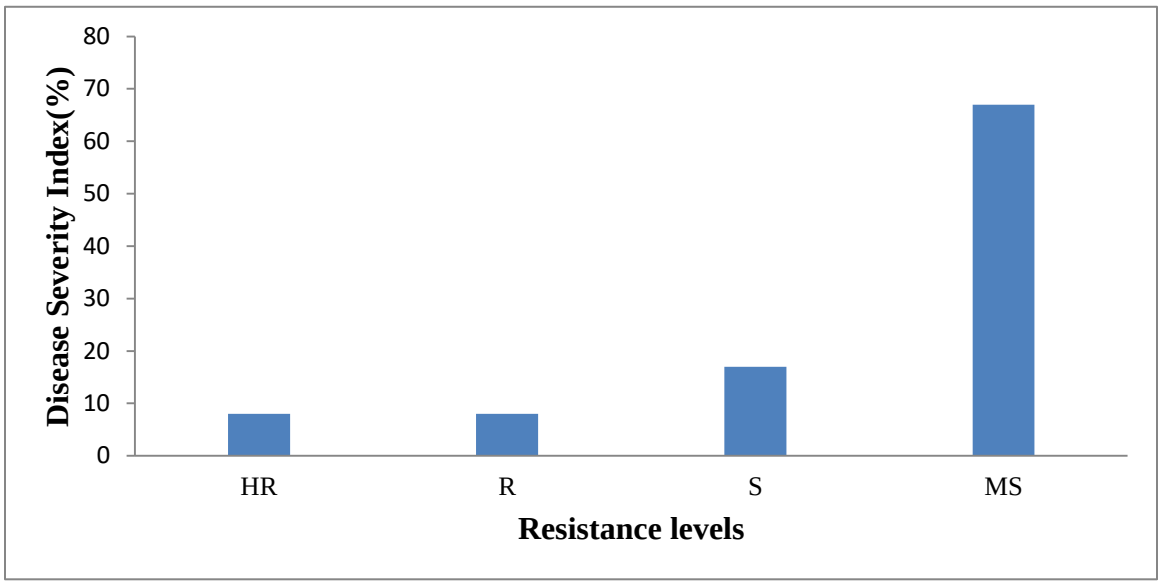

Figure 7: Resistance levels of twelve hot pepper varieties to *Fusarium* wilt disease, based on disease severity index. **Where:** - Highly resistant (HR) = 0%, Resistant (R) = <10%, Moderately Susceptible (MS) = 21-40%, Susceptible (S) =41-60%, DSI= disease severity index.

After 90 days of post infected, the peppers plants uprooted and measured its length and fresh weight. The shoot length of Melka zala was reduced by 56% by Pathogenic *Fusarium* isolates (Fo1), while root length of Dinsire by 61 %. Moreover, shoot and root fresh weights of Melka zala were showed reduction by 61 and 66 %, respectively (Table 8). On the other hand, Melka dera and Marko fana LP less affected by diseases causing *Fusarium* strain (Fo1).

Table 9: Effects of *Fusarium* isolate (FI1) on pepper growth and fresh weight

Varieties	Plant length(cm)						Fresh weight (g)					
	Shoot			Root			Shoot			Root		
	N	I	% Reduction	N	I	% Reduction	N	I	% Reduction	N	I	% Reduction
Marko Fana	69.3	38.3	45	19.7	10.7	54	36.3	18.7	49	8.3	3.5	58
Melka Oli	69.3	50	28	19	12	37	29.7	21.7	27	6.4	3.2	50
Melka Zala	64.7	28.3	56	17.3	7.7	56	30	11.7	61	5.8	2	66
Melka Awze	74.7	54.7	27	19.3	10.7	45	41.2	28.3	31	5.1	3.5	32
Melka Shota	78.3	54.7	30	21.7	11.3	48	39	24.3	38	5.3	2.3	56
Melka dera	76	67.3	11	18.3	15	18	39	37.5	4	8.5	6.5	24
Oda Haro	61	29.3	52	20.3	9	56	32.8	17	48	9.5	5	47
Bako Local	62	48.3	22	21.3	10.7	50	29	21.3	26	7.8	5.7	28
Dinsire	61.7	44.7	28	20.7	8	61	38	24	37	8.3	4.9	41
Dame	59.3	40.7	31	19.3	11.7	40	37.3	21	44	6.9	6	13
Kume	63	50.3	20	19.3	11.3	41	39	25.7	34	6.5	5	23
Marko Fana Large pod	64	54.7	15	20.3	17	16	29.6	30.3	2	9.6	7.7	20

Where: - N= Un-inoculated pepper plant, I= inoculated

Statistical analysis revealed that, all the evaluated pepper varieties significantly reduced the growth parameter. More importantly, there was significant difference among varieties regarding the growth parameter. The varieties Melka Dera and Marko Fana Large Pod show significantly varied from the others.

Table 10: Effects of the *Fusarium* species on growth parameter of the pepper

Varieties	Root length (cm)	Root weight (g)	Shoot height (cm)	Shoot fresh weight (g)
Marko Fana Lp	17.00[a]	7.66[a]	54.66[b]	30.33[a]
Melka Oli	12.00[b]	3.20[e]	50.00[c]	21.66[f]
Dame	11.66[b]	6.00[b]	40.66[f]	21.33[g]
Melka Shota	11.33[cb]	2.33[f]	54.66[b]	24.33[e]
Kuma	11.33[cb]	5.00[c]	50.33[c]	25.66[d]
Marko fana	10.66[cd]	3.46[e]	38.33[d]	18.66[h]
Bako Local	10.66[cd]	5.66[b]	48.33[d]	21.03[f]
Melka Awaz	10.66[cd]	3.50[e]	54.66[b]	28.33[b]
Melka dera	15.00[a]	6.56[a]	67.33[a]	37.53[c]
Oda haro	9.00[e]	5.00[f]	29.33[g]	17.66[j]
Diniser	8.00[f]	4.90[c]	44.66[e]	24.00[e]
Melka zala	7.66[f]	1.96[g]	28.33[h]	11.66[i]
CV	**4.17**	**5.58**	**1.11**	**2.61**
LSD	**0.76**	**0.39**	**0.88**	**0.96**

Where, CV=coefficient of variation, LSD=least significant difference at P<0.05. Means indicated with the same letter are not significantly different; all values in green house conditions represent means of three replicates.

4.4. Antagonistic of *Trichoderma* isolate against *Fusarium* spp.

Results of dual culture indicated, *Trichoderma* showed antagonistic activities in all of the *Fusarium* isolates with different rate of growth inhibition. As incubation time increased from 72 to 168 h, growth inhibition of Fo3 was increased from 16.6 to 100%, while FI7 from 17.7 to 66.6% due to antagonistic *Trichoderma*, However, the growth of FI6 and Fo1 isolates were less inhibited with *Trichoderma* (Table 11 and Fig. 8).

Table 11: Percentage growth inhibitions of *Fusarium* spp.by *Trichoderma* isolates on dual culture (%)

Incubation in hrs	Isolates							
	Fo1	Fo2	Fo3	Fo4	Fo5	FI6	FI7	Fo8
72	4.23	4.23	16.6	3.4	11.76	3.3	17.7	1.96
96	18.51	19.4	48.5	19.65	14.28	10.3	28.5	17.04
120	33.12	26.6	69.32	36.6	20.5	15.38	36.5	32
144	36.47	36.93	85.79	52.38	32.01	24.26	55	45.5
168	48.88	49.6	100	65.01	57	31.5	66.6	60.8
Effectiveness	moderately effective	moderately effective	highly effective	effective	effective	moderately effective	effective	effective

Trichoderma caused the growth inhibition of fungal mycelia and observed as potential isolate (Fig.8).

Figure 8: Dual culture of *Tricoderma* and *Fusarium* isolates on potato dextrose agar that inoculated at same time, green line radius of *Fusarium* isolates in control and treatment, red line radius of *Tricoderma* mycilia in treatment.

5. Discussion

Based on the morpho-cultural characteristics as mentioned by Leslie and Summerell (2006), the pathogens causing wilt in hot pepper were isolated and later confirmed as *Fusarium oxysporum* after macroscopic and microscopic examination. The current study shows that the isolates colony color above produced white and white purple in the revers pigmentation produce brown, white, pale yellow and pink. The pigmentation of *Fusarium* species in culture has shown that the color of the mycelia depends on the species (Leslie and Summerell, 2006). Sultana *et al*. (2014) describe *Fusarium oxysporum* as colony appearance with cottony and thin flat to fluffy (hair) type. The different in color of the *Fusarium* species may be due to the presence of specific pigment produced as a byproduct during mycelia growth and enzymatic activities (Booth, 1971).

Mycelia colony development of *Fusarium* isolates deviated with different culture media and incubation periods. According to Lilly and Barnet (1951), the nutritional requirement of different fungi is different for their growth. This mycelia growth variation on the different media might be due to the varied nutritional content of the medium and the different ability of the isolates in consuming and metabolizing the nutrient component of the PDA and CZA medium. Presence of sucrose in CZA supported higher mycelia growth of the *Fusarium* isolates, as compared to PDA medium, which contained dextrose as a source of carbon. The current study was supported by Naik *et al.,* (2010) who reported that growth of isolates was best on medium containing sucrose found on CZA medium, followed by dextrose, fructose, and maltose respectively. However, Ferniah *et al.,* (2014) have reported potato dextrose agar (PDA) to be the best media for growth of the *Fusarium* isolates and supported a wide range of different fungal mycelia growth because of its simple formulation of dextrose as a source of carbon. According to Paulkar *et al.* (2001), potassium nitrate was found to be the best compound as a source of nitrogen for mycelium growth of *Fusarium oxysporum* f.sp. *ciceris*. In any case, almost all fungi require the same basic element for mycelia growth but differ in their capability of consuming and metabolizing compound that contain the basic elements for mycelia development.

The identification of the *Fusarium* isolates in the present investigation was done on the basis of shape, size, and septation in the macro conidia according to the morpho cultural keys provided by Leslie and Summerell (2006). According to Leslie and Summerell (2006) *Fusarium oxysporum* had thin walled, relatively slender (narrow) and indistinctly septate (generally1- 5

septate) macro conidia, evenly curved fusoid with the widest part in the middle and pointed at both the ends, whereas, *Fusarium solani* had thick-walled, curved, dorsoventrally straight, relatively wider, stout and robust macro conidia in which a large percentage of the conidia had the widest diameter in the upper half of the spore. Cultures of *Fusarium solani* usually were white to cream Color with parse mycelium, whereas, *Fusarium oxysporum* produced floccose (tufts of soft hairs like structure), sparse or abundant mycelia, which ranged from white to pale violet in color. The chlamydospores, when present were formed singly, in pairs, in chains or in clumps (Leslie and Summerell, 2006). The present study also supported by Gupta *et al*., (2010) they reported that the micro conidia of *Fusarium oxysporum*, were abundant, no septate, and oval to ellipsoidal in shape, glass in color. Whereas Macro conidia of the *Fusarium oxysporum* were thin walled, delicate, 3-5 septate, cylindrical, straight to curved, sickle-shaped and pointed at both the ends. Sultana *et al.* (2014) had similar reports that support the findings of this study.

The size of micro conidia and macro conidia differing in dimensions between the *Fusarium* isolates. Nirmaladevi and Srinivas (2012) reported that the length x width of the macro conidia in *Fusarium oxysporum* differ from in the range of 15.0-37.5 x 2.5-4.0 µm and the size of the micro conidia among the isolates differ from in the range of 2.5-15.0 x 2.0-3.0 µm. Similar results have been reported by Joshi *et al.* (2013) they also found that the size of macro conidia of *Fusarium oxysporum* was in the range of 5.3-28.4 µm and the size of micro conidia differ from 2.3-11.8 µm. In like manner to the current result, Ferniah *et al*. (2014) reported that the macro conidia was a straight, fusiform, spiny-finned basal cell, and the length x width of the macro conidia in the range of 27.0-46.0 x 3.0-4.5 µm in size with 3-5 septations, whereas, the micro-conidia were abundant, ellipsoid or fusiform, and the length x width of the micro conidia in the range of 5.0-15.0 x 2.2-3.5 µm in size. These differences in the conidial morphology might be due to the age, the flexibility and the effect of different external factors such as chemical composition, concentration and reaction of substrate, temperature, humidity, light, pH, etc., as reported by Tshilenge *et al*. (2010).

The pathogens in the present study showed variation in the temporal expression of the disease symptom. The disease symptom included combinations of curvature of leaves, wilting, chlorosis, shedding of leaves followed by upward and inward rolling of the upper leaves and subsequently wilting of the plant. These isolates were pathogenic to pepper, which caused symptoms typical of

Fusarium wilt. Based on these observations, it was confirmed that *Fusarium oxysporum* were pathogenic to pepper, which has also been supported by the findings of Naik (2006) who after doing the detailed studies of the diagnostic symptoms of wilt in pepper. The variability in these isolates might be brought about by either natural mutation, natural selection, genetic drift, gene flow or mating system (McDonald and Linde, 2002).

Kaur *et al.* (2015) who reported that the isolate designated by Foc-8 in chickpea was more virulent (98.48%), whereas, isolate designated by Foc-24 was least virulent (7.22%). Ferniah *et al.* (2014) also reported that the *Fusarium oxysporum* isolate designated by P1a was highly pathogenic that caused wilting in two chili cultivars TM999 and Gantari with 40 and 63 percent disease severity, respectively. Similarly also Nirmaladevi and Srinivas (2012) determined highly pathogenic (18 isolates), moderately pathogenic (40 isolates), weakly pathogenic (20 isolates) and non-pathogenic isolates of *Fusarium oxysporum* based on the characteristic wilt symptoms in the tomato cultivars. The appearance of specific symptoms differs depending on the virulence/harmfulness level of a specific isolate of *Fusarium oxysporum*. The lacking uniformity in these isolates might be brought about by either natural mutation, natural selection, genetic drift, gene flow or mating system (McDonald and Linde, 2002). The severe pathogenic behavior of isolate Fo1 and Fo5 in present results is might be due to the high efficiency in spore and mycelium production.

The study was undertaken to identify wilt resistant pepper varieties at JUCAVM greenhouse. Specifying of varieties on the basis of their reaction to *Fusarium* wilt diseases make easier for the identification of resistant and susceptible varieties based on disease severity index percent and severity rating scale. The present study is in agreement with the reports of Mamta *et al*.,(2015) which indicated great disease incidence variation within the range of 0 to 78.7% and from thirty varieties screened, only two varieties were found 100% resistant, against wilt of pepper. In the same way the current study is in agreement with the reports of Aklilu *et al.*, (2018) which indicated great disease incidence variation within the range of 0 to 100% and from fifty four hot pepper genotype screened; only two varieties were found resistant, against wilt of pepper.

Based on incidence percent from the total of twelve pepper varieties screened, two varieties were found susceptible with DSI (40-60%), eight were moderately susceptible (21-40%), and one

variety resistant (8.8%) and the other one variety were rated highly resistant (0%). Wide difference in resistance scores rating scale was observed between different pepper varieties which are due to their genetic difference among the pepper cultivars. The resistance variety may be due to their genetic background with higher metabolic/gene activity unsuitable to the *Fusarium* wilt pathogen. The potential of the varieties to produce phytoalexins also influence their resistance to fungal infections. According to Sahi *et al*., 2000 and Iftikhar *et al*., 2005 the reason for resistance might be due to antifungal compounds such as phenolics produced by resistant variety that was more potent than other compounds, especially those produced by susceptible varieties, thus, it must have a role in imparting resistance against *Fusarium* wilt disease. The compounds such as phenolics produced only by *Fusarium* wilt resistant varieties.

The main problem in screening varieties is that some varieties found resistance at one location turns out to be susceptible to another place. Therefore, environmental-gene interaction has a major role for very long lasting resistance (Ashfaq *et al*., 2008). The genetic structure of a plant also can affect the resistance of plants against the fungus in addition to environment and amount of fungus inoculating. The difference of the resistant/moderately resistant varieties with that susceptible varieties indicated that resistance could either delay the initial infection of the disease or slow down the rate of wilting.

The use of resistant variety is beneficial not only in reducing losses due to diseases but also useful to minimize the fungicidal toxicity (Manu *et al.,* 2014). These identified varieties could be used in hybridization programs to develop varieties possessing desirable traits besides resistance to *Fusarium* wilt pathogen. However, it would be too much to expect stable resistance against *Fusarium* diseases because of high variability and dynamic nature of the pathogen (Devika Rani *et al.,* 2008). Hence inherent resistance or tolerance of crop plants to infection by the pathogen can most likely to be a safe, most economical and ecofriendly disease management practice (Parey *et al*., 2013). The inheritance of *Fusarium* wilt resistance in pepper has been of monogenic dominance in nature; hence, the crossbred organism is advocated to boost the yield. Thereby avoiding the application of pesticides and avoiding environmental pollution (Manu *et al*., 2014).

In the present study, the reduction in total plant length and in total plant fresh weight in each plant that inoculated by *Fusarium oxysporum* were appeared. These were probably the isolate of

Fusarium oxysporum once enter into the plant root system, block the line in which the plants necessary material like water, minerals, and food were transported. If the plant doesn't get enough water and mineral for growth it becomes chlorosis (yellow), wilted and reduced in growth parameter (length and weight). This was supported by Rakesh *et al.*, (2017).

The different findings showed that *Trichoderma harzianum* has an inhibitory effect on the mycelia growth of *Fusarium oxysporum* in all the treatments in the dual culture. Srideepthi and Krishna (2015) reported that efficacy of *Trichoderma harzianum* against *Fusarium oxysporum* f.sp. *capsici*. The result also shows that the *Trihoderma* isolates inhibit the growth of *Fusarium oxysporum.* These the faster growth with higher inhibition of the fungus was might be due to producing extracellular lytic enzymes and competition with *Fusarium oxysporum* for nutrients and space which arrested the growth of *Fusarium oxysporum*. The rapid growth and competition for nutrient and space by the antagonist inhibited the growth of the pathogen. The pathogen presented a defined edge of growth opposite to the antagonist, with lysis occurring in the mycelial fragments in the contact zone. Siameto *et al*., (2011) reported that the produced enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of *Fusarium oxysporum* cell walls, thereby destroying the cell wall integrity and limiting the growth of *Fusarium oxysporum* toward the antagonist strain such as *Trichoderma* isolates. In all the treatments, the antagonist overgrew the pathogen resulting in starvation and subsequent death of the pathogen. Suprapta, 2012 and Ekefan *et al*., (2009) had similar reports that support the findings of this study.

6. Conclusion

Based on the current result the following conclusions were made.

The *Fusarium* isolates that were identified from pepper root samples able to caused *Fusarium* wilt disease on hot pepper varieties. Among the eight identified *Fusarium* spp. based on severity index and AUDPC results, one isolates (Fo1) was identified as the most aggressive, two weak, and the rest observed as moderately pathogenic to the tested hot pepper. Potential peppers growing areas of Jimma zone are infested with pathogenic *Fusarium* species. Melka Dera and Mareko Fana large pod showed resistance to pathogenic *Fusarium* and most of varieties were recorded as moderately susceptible. Melka Dera considered as promising variety against *Fusarium* wilt. The resistance level of Ethiopia hot peppers varieties against *Fusarium* diseases is low. *Tricoderma* sp. was effectively inhibited the radial growth of all *Fusrium* isolates.

7. Recommendation

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

- The conventional methods of identification of the isolates cannot show the exact identity and true diversity. Thus, molecular approach will be overcome this drawback.
- The genes responsible for virulence of *Fusarium* isolates and resistance of Pepper varieties will be further investigated. And also further screening of pepper genotypes, varieties and accession against *Fusarium* diseases will carry out.
- The efficacy of *Trichoderma* sp. will be evaluated under greenhouse and field conditions

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9. Appendices

Appendix A: Analysis of Variance (ANOVA) Tables

Appendix Table 1 ANOVA table for mean squares for the effect of *Fusarium* spp. on growth parameter of pepper at probability level of 0.05

		Pepper growth parameters							
Source	**DF**	**RL***		**RW***		**SH***		**SFW***	
		Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F
Rep	2	0.0833^{ns}	0.6703	0.1219^{ns}	0.1299	0.6944^{ns}	0.0993	0.1111^{ns}	0.7128
Var	11	17.1212**	<.0001	8.2665**	<.0001	276.080**	<.0001	132.808**	<.0001
Error	22	0.2045		0.0543		0.2702		0.3232	
Total	35								
CV		4.1747		5.5886		1.1112		2.6106	
LSD		0.7658		0.3948		0.8802		0.9627	

*RL=Root length, RW=Root weight, SH=Shoot height, SFW=Shoot fresh weight, ^{ns}=Non significant, **=highly significant

Appendix B: List of Table in the Appendix

Appendix Table 2: Place of Collection of pepper varieties/cultivars

No	Pepper cultivars/varieties	Place of collection(center/institute)
1	Mareko fana	Melkasa Agricultural Research Center
2	Melka oli	Melkasa Agricultural Research Center
3	Melka zala	Melkasa Agricultural Research Center
4	Melka awaz	Melkasa Agricultural Research Center
5	Melka shota	Melkasa Agricultural Research Center
6	Melka dera	Melkasa Agricultural Research Center
7	Oda haro	Bako Agricultural Research Center
8	Bako local	Bako Agricultural Research Center
9	Dinsire	Bako Agricultural Research Center
10	Dame	Bako Agricultural Research Center
11	Kume	Bako Agricultural Research Center
12	Mareko fana large pod	Bako Agricultural Research Center