DISTRIBUTION AND MANAGEMENT OF FUSARIUM WILT (*Fusarium oxysporum* f.sp. *lentis)* OF LENTIL (*lens culinaris* MEDIKUS) IN CENTRAL HIGHLANDS OF ETHIOPIA

MSc THESIS

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Distribution and Management of Fusarium Wilt (*Fusarium oxysporum* f.sp. *lentis)* of Lentil (*Lens culinaris* Medikus) in Central Highlands of Ethiopia

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APPROVAL SHEET

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We hereby certify that we have read and evaluated this Thesis entitled **'Distribution and Management of Fusarium Wilt (*Fusarium oxysporum* f.sp. *lentis)* of Lentil (***Lens culinaris*** Medik**us) in Central Highlands of Ethiopia'** prepared under our guidance by **Tolesa Bedasa Abdisa.** We recommend that it be submitted as fulfilling the Thesis requirements.

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DEDICATION

This work is dedicated to my lovely parents.

STATEMENT OF AUTHOR

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
CSA	Central Statistical Agency
DAP	Days After Planting
DZARC	Debre Zeit Agricultural Research Center
FAO	Food and Agriculture Organization of the United Nations
FW	Fusarium Wilt
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
m.a.s.l.	meters above sea level
PDI	Percent Disease incidence
PCE	Plant Count after Emergence

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ABSTRACT

*Fusarium wilt of lentil (*F. oxysporum f.sp. lentis*) causes huge lentil yield losses in central highlands of Ethiopia. In this study, extensive wilt survey of eight major lentil-growing districts, viz. Adaa, Aleltu, Lume, Gimbichu, Minjar-Shenkora, Siyadebrena Wayu, Moretina-jiru and Ensaro in central highlands of Ethiopia was conducted during the 2017cropping season. The study of the objectives were to: 1) assess the distribution of lentil Fusarium wilt in the central highlands of Ethiopia; 2) determine the extent of seed infection due to lentil Fusarium wilt pathogen on seed lots collected from different sources in the central highlands of Ethiopia; and 3) evaluate the effect of lentil varieties and seedbed types as components of integrated management option. Data of the survey revealed 100% mean wilt prevalence and 32.8% mean wilt incidence were observed. The highest (75%) and the lowest (2%) wilt incidence were recorded in Moretina-jiru and Lume, respectively. Morphological and cultural assessment of 192 isolates of wilt causal agents showed hihgest frequencies of* Fusarium oxysporum *f.sp.* lentis *(100%) followed by* Rhizoctonia *spp. (23.4%) and* Sclerotium rolfsii *(3.0%). A factorial experiment involving lentil variety and seedbed type, each at four levels, was carried out in a split-plot design with three replications. The four lentil varieties were ILL-590 (susceptible check), Alemaya, Derash and Denbi, and four seedbed types were flat bed, open raised bed, tie-raised bed and farmer's practice. Among the seedbed types, raised seedbed exhibited relatively lower disease incidence than others. Interaction of the varieties and seedbed types was significant in wilt reduction. The highest wilt incidence (ca. 82.0%) was recorded on ILL-590, susceptible lentil line, planted on flat bed; whereas, the lowest (ca. 8.8%) Fusarium wilt incidence was noted on cv. Derash planted on raised bed. A combination of cv. Derash and raised bed gave significantly ($P \leq 0.05$) higher grain yield (3827.0 kg/ha) than all other treatment combinations at Chefe Donsa. Unlike Chefe Donsa, raised bed contributed significantly ($P \leq 0.05$) higher grain yield of not only cv. Derash but also cv. Alemaya than all other treatment combinations at Debre Zeit. Significantly ($P \leq 0.05$) lower grain yields (in the order of 68.0 kg ha^{-1}) were obtained from integration of the susceptible genotype (ILL-590) with flat bed than all other treatment integration irrespective of the trial locations. The highest (1018.0% unit/days) in AUDPC values were obtained by flat seedbed type and ILL-590, while the lowest (342.4% unit/days) in AUDPC values were obtained by raised seedbed type and Derash variety at Debre Zeit. Wilt incidence and AUDPC values were significant and negatively correlated with yield parameters. Lentil seed infection due to* Fusarium oxysporum *f.sp.* lentis *ranged from 0 to 22.2%. It was concluded that using resistant variety with raised seedbed significantly reduced* Fusarium *wilt incidence and gave reasonably high yields. The future research work should focus on screening several lentil genotypes for source of resistance.*

Keywords: Lentil, Survey, Management, Wilt, Fusarium oxysporom f.sp.lentis

1. INTRODUCTION

Lentil (*Lens culinaris* Medikus) is a high value cool season pulse crop and contains about 25% protein in its seeds (Zia *et al*., 2011). Its production is concentrated in the northwest provinces of Australia, Bangladesh, China, Ethiopia, India, the Middle East, Nepal, North America, Syria and Western Asia (Abraham, 2015). Ethiopia is the leading producer of lentil in Africa, followed by Morocco and Tunisia and is seventh in the world (Abraham, 2015). Its total area and production in Ethiopia is about 113,684 ha and 0.17 million tons, respectively, with an average yield of 1.5 tons ha^{-1} (CSA, 2016). The major lentil-producing regions in Ethiopia are Oromia, Amhara, Tigray and the Southern Nations, Nationalities and Peoples' (SNNP) (Senait *et al*., 2006).

Lentil is mainly grown in the highlands of Ethiopia during the main and small rainy seasons (Jarso *et al*., 2009). Lentil planting date varies from region to region and it usually ranges from late June to Mid July in both mid and high altitude areas, depending on the amount and distribution of rainfall, temperature, topography and elevation of the areas where there is not waterlogging problem (Senait *et al*,, 2006). In the waterlogged highlands, planting is done when the rain stops in later September and early October. A soil pH of 6-8 is conducive for lentil production, but it can also tolerate a moderate alkalinity (Mulugeta, 2009). It requires a minimum of 500 mm rainfall and a maximum of 850 mm; in the higher rainfall areas, good drainage is essential since waterlogged soil will have a great negative effect on yields and can aggravate disease development (Getahun, 2016). That means the crop plant is highly susceptible to excessive moisture stress (Mulugeta, 2009). Waterlogged soil limits the oxygen diffusion of the soil and, as a consequence, crops become more susceptible to plant diseases (McDonald and Dean, 1996). Therefore, cultural practices significantly affect the growth of crops as well as their yields due to the changing soil structure and moisture during the vegetative growth period.

Lentil plays a significant role in human and animal nutrition and in maintenance and improvement of soil fertility (Frederick *et al*., 2006; Sarker and Kumar, 2011). Lentil has a very good potential for increasing farm income (Das *et al*., 2013). Farmers and their families use it to make the local *Nifro* (boiled lentil), *Sambusa* (boiled whole lentil that is roasted in oil after wrapping with paste of wheat flour), and *Shorba* (soup) and *wot* [local soup for moistening and eating along with *Injera* (flat pancake) or bread]. Ethiopian farmers' produce the lentil crop

mainly for food, cash income, and more importantly to restore soil fertility and the straw is excellent for animal feed (Altaf *et al*., 2014).

The yield of lentil remains low (1.5 tons ha^{-1}) in Ethiopia (CSA, 2016) and still relatively low compared to its yielding potential (3.6 tons ha^{-1}) under well managed production due to biotic and abiotic stresses (Kumar *et al*., 2017). This low lentil production is attributed to various diseases, insect pests, poor agronomic practices, and lack of improved cultivars and crop protection technologies (Ghazanfar *et al*., 2010). The crop suffers from various plant diseases, which are caused by fungi, viruses, nematodes, and parasitic weeds and insect pests thereby resulting in huge economic losses (Khare *et al*., 1979). In Ethiopia, lentil wilt/root rot complex caused by *Fusarium* spp, *Rhizoctonia solani, R.bataticola, Sclerotium rolfsii,* Ascochyta blight (*Ascochyta lentis*) and rust (*Uromyces viciae fabae*) are the most important biotic factors causing lentil yield reductions (Ahmed and Ayalew, 2006; Negussie *et al*., 1998).

Lentil Fusarium wilt (FW) (*Fusarium oxysporum* f.sp. *lentis: Fol*) plays a major role in reducing lentil yield (Pouralibaba *et al*., 2015), and causes severe damage to leaves, stems, roots and pods (Singh, 2015). This pathogen can cause infection at all stages of plant growth with more incidences at flowering and podding stages than early vegetative stage (Chavdarov, 2006). Under field conditions, the typical wilting can appear within three to four weeks after sowing, if the variety under consideration is susceptible (Taylor *et al*., 2007).

Fusarium wilt, which is a vascular fungal disease, is the most devastating of all lentil diseases worldwide that can cause extensive yield losses reaching up to 100% in prolonged favorable environments (Kumar *et al*., 2010). It is considered as the most damaging soilborne disease of lentil and is a major lentil production-limiting agent worldwide (Tosi and Cappelli, 2001). The damage also depends upon the crop stage being affected, aggressiveness of the pathogen strain and disease severity (Taylor *et al*., 2007). Fusarium wilt ocuurs as a complexs with other root roting pathogens causing severe lentil seedling losses (Schneider *et al*., 2001; Hamwieh *et al*., 2005). Damping-off diseases, soilborne diseases caused by *Rhizoctonia solani* or *Pythium* spp., (Wang *et al*., 2003).

Lentil is mainly produced on vertisols characterized by waterlogged conditions due to high rainfall in the highlands of Ethiopia (Jutzi, 1988). Vertisols are characterized by severe

waterlogged soil during the rainy season due to its expansion, flaking and crust formation characteristics that reduces its percolation rate (Deckers *et al.,* 2001). Excess soil moisture and waterlogged soil creates an environment conducive for the development of wilt/root rot pathogen (Midmore, 2015). This cause breakdown of host resistance to Fusarium wilt of lentil, probably through retarding phenylalanine ammonia lyase (PAL) enzyme(s) activity (Midmore, 2015).

Poor germination in soils at or near saturation has been related to limited oxygen diffusion through thick water films surrounding the seed (Richard and Guerif, 1988). The availability of water must be balanced with aeration potential to meet requirements for germination and crop establishment (Dasberg and Mendel, 1971). Interactions between high soil temperature and excessive water content can promote fungal attack on the seed (Woodhead, 1987). The permeability of the roots to water is reduced the oxygen levels, and eventually the roots lose their ability to control water movement (Braunack and Dexter, 1989). Climate change has been one of the greatest economic, social and environmental threats and predicted that agriculture shall join economic sectors that face the biggest harmful effects (Redman *et al*., 2010).

Okada *et al.* (1991) also postulated that persistence of wetness within rooting zone adversely affects the crop growth since legumes are sensitive to excess moisture. Several attempts that have been directed to minimizing or limiting the pathogen effects on plants by seedbed preparation practices are quite rare for legumes, particularly for lentil. Fusarium wilt incidence is influenced by the amount of stress the plant is exposed to soil compaction, poor drainage and resultant excess moisture, low soil fertility, low pH, and temperature extremes that exacerbate root rot severity (Awadhawal *et al*., 2001). Inadequate seedbed preparation contributes to favorable condition for Fusarium wilt pathogen. To avoid this problem, a set of appropriate seedbed type is very important to improve the soil physical conditions and well drainage excess soil moisture. Solution for this and similar other problems can be realized by cultivating improved lentil crops on appropriate seedbed to make adequate drainage in soils with excess soil moisture to prevent root diseases (Feiza *et al.,* 2010).

Fusarium wilt/root rot disease management in lentil is critical and relies heavily on integrated management. However, none of the control measures are found to be effective and adequate individually at field level (James and Pandey, 2017). A number of lentil FW resistant varieties had been identified at national and international levels to manage this risk through the use of

wilt-sick plot technique. And also efforts have been made to check the potential of existing germplasms as well as to develop high yielding varieties with desirable characters. However, due to pathogenic variability and new virulent forms of the pathogen, the host plant resistance is not stable because most of the varieties that were formerly resistant have become susceptible today. Thus, integration of host plant resistance with cultural practices against the prevalent pathogen should be considered as management options against lentil Fusarium wilt in Ethiopia.

Currently, lentil Fusarium wilt was increasing in intensity and distribution in lentil-growing areas of Ethiopia. Although wilt/root rot tolerant lentil cutivars were released in Ethiopia, the losses from soil borne diseases were high in some years where there is high amont of rainfall. However, not much study on disease mangment, pathogens associated with infected plants in different time of planting and seed infection level lentil by *Fol* were not done in Ethiopia.Thus, an integrated disease management approach was suggested to be essential to combat Fusarium wilt of lentil for increased and sustainable yields.

Therefore, the objectives of the study were to:

1) Assess the distribution and importance of lentil Fusarium wilt in the central highlands of Ethiopia;
2) Determine the extent of seed infection due to lentil Fusarium wilt causing pathogen on seed lots collected from different sources in the central highlands of Ethiopia; and
3) Evaluate the integration of lentil varieties with seedbed types as components of integrated management option against Fusarium wilt epidemics and lentil yield.

2. LITERATURE REVIEW

2.1. Distribution of Lentil

Lentil (*Lens culinaris* Medikus) is one of the world's oldest domesticated leguminous crops, (Zohary and Hopf, 2000). Cultivated lentil is thought to have been originated and first domesticated in western Asia and then introduced into the Indo-Gengetic Plain around 2000 B.C. (Cubero, 1981). Lentil has also been rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later to the new world, including Latin America, Mexico Chile, Argentina, Colombia and more recently Canada, and cultivated in most sub-tropical and warm temperate regions (Abraham, 2015). Lentil remains stable food crop in the Middle Eastern and Indian diets, and one popular in the cuisines throughout the world (Anonymous, 2013).

Lentil is a short and slender annual cool-season food legume, which was domesticated early in the Fertile Crescent of the Middle East (Sarker *et al*., 2010). The botanical features of lentil (*L. culinaris*) can be described as annual bushy herb with slender almost erect or sub-erect, much-branched, softly hairy stems that are slender angular, 15-75 cm in height and show hypogeal types of germination (Muehlbauer *et al.,* 1997). It is a brushy annual plant of the legume family, grown for its lens-shaped seeds, which produces many small purse-shaped pods containing one to two seeds each. Lentil is classified into two groups by seed size, namely the Chilean and Persian types. The large seeded Chilean type has 1000 seed weight of 50 g or more. The small seeded Persian type has 40 g or less of an average weight per 1000 seeds.

Lentil has been considered to be the poor man's meat due to an affordable other sources of protein. About one-third of the calories in lentil come from protein, which is the third-highest level of protein by weight of any legume. In many parts of the world, lentil is the cheapest protein food and contains dietary fiber, vitamin B and minerals, iron, among the cool season legume crops, lentils are the richest in their important amino acids (lysine, arginine, and leucine) contents; however, there is shortage of certain lentil amino acids, including methionine and cystine (Muehlbauer *et al*., 2002). Lentil is important, especially for women of child-bearing age, children and vegetarians. The crop has great significance in cereal-based cropping systems because it fixes nitrogen and the straw provides animal feed (IBC, 2007; Muehlbauer, 2011).

The Lentil Improvement Program of the Ethiopian Institute of Agricultural Research (EIAR) in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) has released some lentil varieties; previously these varieties were resistant to Fusarium wilt and had yield potentials of up to 2.6 t ha^{-1} (Mulugeta, 2009). According to FAO (2009) production database, currently Ethiopia contributes to 2% of the world total production, and it was the first producer of lentil in Africa accounting to 84% of the total regional production (96,524 tons), followed by Morocco (8.8%), Malawi (1.9%), Egypt (1.6%) and Tunisia (1.2%). Currently, lentil is considered as a cash crop that fetches higher price than most of the cereals and pulses grown in Ethiopia (Abraham, 2015).

2.2. Production and Consumption of Lentil

Lentil provides an important source of food and nutritional security for the rural poor, especially those who cannot produce or cannot afford costly livestock products as source of essential proteins. Lentil is recognized as one of the most nutritious pulse crops ranking next to chickpea amongst pulses. It contains 24-26% protein, 3.2% fiber and 57% carbohydrate. It is a rich source of minerals containing 68 mg calcium, 300 mg phosphorus and 7 mg iron per 100 g lentil seed. It is also rich in vitamin C and riboflavin (Ali and Mishra, 2000). Furthermore, because of its high lysine and tryptophan contents, its consumption with wheat or rice provides a balanced diet. The chemical composition and nutritive values of lentil haulm vary with variety, soil, climatic conditions, sowing date, stage of harvest and storage conditions (Demirel *et al.*, 2012).

The major lentil-growing countries of the world include Australia, Canada, China, Ethiopia, India, Nepal, Syria, Turkey and USA (Ahlawat, 2012). The total lentil cultivated area in the world is estimated at around 4.34 million hectares with annual production and productivity of 4.95 million tons and 1260 kg ha^{-1} respectively (FAO, 2014). Most of the production, which reaches around 56%, is consumed locally and only 44% of the production is supplied to the global markets (Kumar *et al.,* 2013). Canada is the leading lentil exporting nation worldwide (Bedard *et al.,* 2013).

Among grain legumes, lentil is one of the principal crops widely grown in diverse agro-ecological zones of Ethiopia (Muehlbauer and Tullu, 1997; Schneider and Anderson, 2010). Food legumes are grown throughout Ethiopia and account for 13% of the cropped land that is

concentrated in the Amhara and Oromia regions (Rashid *et al.*, 2010). The country's potential lentil producing zones are six zones of Amhara region: North Gondar, North Shewa, North Wollo, South Gondar, South Wollo and Waghemra; three zones in Oromia region are East Shewa, North Shewa and Southwest Shewa, while South Tigray is the major lentil-producing zone in the Tigray Region (Senait *et al*., 2006). The average seed yield of lentil in farmers' fields in Ethiopia generally ranges from 0.6 to 0.8 t ha^{-1} (Senait *et al*., 2006). Not surprisingly, lentil yield that was restricted to about 2.0 t ha^{-1} has been reported from experiments performed under controlled field experimental conditions in Ethiopia (Teklu *et al*., 2006).

2.3. Lentil Improvement in Ethiopia

Lentil is one of the major highland pulses of Ethiopia that grow in rotation with barley, tef, and wheat, particularly on the heavy black clay soils (vertisols). Lentil research in Ethiopia was started in 1972 at Debre Zeit Agricultural Research Center (DZARC), which is now the National Lentil Improvement Program Coordinator and has released lentil varieties, namely E1-142, R-186, Chalew (NEL-358), Chekol (NEL-2704), Gudo (FLIP 84-78L), Adaa (FLIP 86-41L), Alemaya (FLIP 88-63L), Alem Tena and Teshale. Among these EL-142, Chekol and Alem Tena were released for the lowland dry areas. Varieties R-186, Chalew, Gudo, Adaa and Alemaya were released for the central, northern and southeastern highlands of Ethiopia (Bejiga and Anbessa, 1998). Kayan and Olgun (2012) reported that hundred seed weight and grain yield in lentil in Ethiopia increased from 0.5 to 0.6 g per 100 g and from 0.5 to 0.6 t ha^{-1} in landraces from 4 to 5 g per 100 g and from 3.0 to 3.5 t ha^{-1} in improved varieties through research efforts (Hassan *et al*., 2009).

2.4. Agro-Ecological Distribution of Lentil in Ethiopia

Lentil is one of the principal cool-season food legumes widely grown in diverse agro-ecological zones of Ethiopia (Joseph *et al*., 2014). It is grown as a main season crop and is particularly important in Amhara and Oromia Regions, and some parts of Southern Nations, Nationalities and Peoples' Region and Tigray Region (Lijalem, 2009; Wang, 2012). It is one of the less selective legumes in climate and soil features and preferences (IBC, 2007). It is usually well adapted to various soil types ranging from sand to clay loam when there is good internal drainage (Joseph *et al*., 2014). Lentil appears very sensitive to waterlogged field conditions and even short

period of exposure to excessive soil moisture can cause the crop to succumb to waterlogging easily (Schneider and Anderson, 2010).

Lentil is widely grown in areas having an altitudinal range of 1700-2400 meters above sea level (m.a.s.l.) with annual rainfall ranging from 700-2000 mm in Ethiopia (Lijalem, 2009; Wang, 2012). It is also well adapted to various soil types and performs best on deep, sandy loam soils with high phosphorus and potassium contents but very sensitive to waterlogged conditions, even slight exposure of flooded field can cause severe destruction of the crop. To alleviate these problems, farmers should use appropriate land preparation (ridge bed) to ensure adequate water drainage, adjust planting date to the right time, use resistant variety, and design proper tillage practices. Soil tillage practice significantly affects the growth of crops as well as their yields within the changing soil structure and well-drained moisture during the vegetative period. The different sowing dates, genotypes, cultivation years, locations, and their interactions have highly significant effects on grain yield and aboveground total biomass of lentil (Wang, 2012). Lentil is grown in the short rainy season ('belg', February-May) in Ethiopia and during the main rainy season ('kiremt', June-December), the latter period being predominant in the country.

2.5. Major Biotic Constraints to Lentil Production

Currently, the consumption of lentil is decreasing mainly because of the high level of limiting factors on its production and productivity in Ethiopia. The yield-limiting factors include cultivation of local varieties for long period, inappropriate seedbed type, poor field management, use of unhealthy seed, lack of lodging resistance, lack of seedling vigor, high rate of flower drop, low pod setting, poor dry matter, low harvest index, low or no response to inputs, and subject to various biotic and abiotic stresses. In Ethiopia, there are about ten important lentil diseases, among which Fusarium wilt, root rots and rust are the major ones (Abraham, 2015). Fusarium wilt is the most important biological constraint to lentil productivity worldwide, causing severe damage to leaves, stems, roots and pods as well as seed quality reduction (Garkoti *et al.*, 2013). Singh (2015) reported that lentil is attacked by a number of seed- and soil-borne pathogens, such as vascular wilt (*Fol*), root rot (*Rhizoctonia solani*), dry root rot (*R. bataticola*), downy (*Peronospora lentis*) and powdery (*Erysiphe pisi*) mildews, rust (*Uromyces vicia-fabae*), and collar rot (*Sclerotium rolfsii*).

2.6. Impact of Fusarium Wilt on Lentil Production in Ethiopia

Taylor *et al.* (2007) reported that root diseases are generally most severe where susceptible lentil varieties are grown in short cropping rotation, because the pathogen inoculum can build up quickly when environmental conditions are conducive for disease development (Est´evez *et al*., 2002; Abawi and Ludwig, 2005). Fusarium wilt (*Fol*) is globally recognized as the most important factor in reducing and limiting lentil production (Erskine *et al*., 2009). The disease may cause complete crop failure under favorable conditions for disease development, and can be the major production limiting factor for lentil cultivation in certain areas (Chaudhary and Amarjit, 2002). The fungus *Fol* is primarily a soilborne pathogen. However, few reports indicate that it can be transmitted through seeds (Haware *et al*., 1990). Lentil wilt epidemics can occur during the early stage of crop cycle; however, the wilt injury can take place at any developmental stage of the plant under various climatic conditions (Turk *et al*., 2004; Abd-Allah and Hashem, 2006). Hwang *et al*.(2001) indicated that Fusarium wilt causes damping-off, seedling blight, root rot, and reduces stand establishment, nitrogen fixation, root distribution, and root vigor.

2.7. Distribution of Lentil Fusarium Wilt and Yield Losses due to the Disease

Kumar *et al*. (2010) reported that the vascular wilt of lentil has widely distributed with reports of its occurrence from as many as 26 countries worldwide. The disease was first reported from Hungry (Fleischmann, 1937). Later on, the disease had been reported from many other countries, including Czechoslovakia, Egypt, Ethiopia, France, India, Myanmar, Nepal, Pakistan, Syria, Turkey, USA and USSR (Hulluka and Tadesse, 1994). In a recent study, 12 fungal species were isolated from the diseased lentil plants collected from different states of India with *F. oxysporum* f.sp. *lentis* as the major pathogen (30%), followed by *Rhizoctonia bataticola* (17.5%) and *Sclerotium rolfsii* (15.7%) (Chaudhary *et al*., 2009). Fusarium wilt is known to cause serious economic yield losses in some parts of the world (Chaudhary *et al.*, 2009, 2010).

According to Garkoti *et al*. (2013), yield losses depend on the stage, where the plant wilt can be 100% when wilt occurs at seedling and pre-pod stages, whereas under adult about 67% when it occurs at flowering and podding stages, while the pre-harvest stage plant infections are able to produce some grain yields. The disease is estimated to cause economic yield losses in parts of South America, South Asia, Sub-Saharan Africa (SSA) and West Asia and North Africa

(WANA) region (Erskine *et al*., 1994). Fusarium wilt causes yield losses up to 50% in India. In Madhya Pradesh, for instance, wilt incidence as 50-78% has been reported in some fields (Khare *et al*., 1979). The yield losses vary from 5-12% and may reach 72% in Syria and a complete crop failure in India (Khare, 1981; Bayaa *et al.*, 1986). Average loss due to lentil vascular wilt in Algeria was estimated at 10% but can reach 66% (Belabid *et al*., 2000).

2.8. Nomenclature, Taxonomy and Morphology of *Fusarium oxysporum*

Agrios (2005) reported that the genus *Fusarium* belongs to the class Fungi Imperfectii (Deuteromycetes or mitosporic fungi). It comprises of many species and many forms within species. *Fusarium* species were traditionally classified in the Deuteromycotina/Fungi Imperfectii although affinities to Ascomycotina have been established. Traditional classification and identification schemes for Fusarium are exclusively based on a morphological species concept derived from cultural characteristics of single-spore isolates grown on special media, shared morphological trait of the anamorph, host range and, to a lesser extent, teleomorph micromorphology (Booth, 1971). According to Agrois (2005) the pathogen is classified as follows: Kingdom: Mycota, Division: Eumycota, Sub-Division: Deuteromycotina, Class: Hyphomycetes, Fungi Imperfect, Order: Moniliales, Family: Tuberculariaceae, Genus: *Fusarium*, Form species: *Fusarium oxysporum* f.sp. *lentis*.

2.8.1. Pathogen

Fusarium is among the most diverse genera and very important pathologically (Synder and Hansen, 1989). *Fusarium oxysporum* is an asexual fungal species that includes human and animal pathogens and a diverse range of nonpathogens. Pathogenic and nonpathogenic strains of this species can be distinguished from each other with pathogenicity tests. No sexual stage is yet known for *F. oxysporum.* The fungus has septation and profusely branched growth on potato dextrose agar (PDA) at 25 ºC (Booth, 1971). Initially it produces white mycelial growth turning light buff or deep brown later and fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, whitish/cream, dark purple, light orange) may be observed in culture on solid medium. The color of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink or purple and on the reverse, it may be colorless, tan, red, dark purple, or brown (Kontoyiannis *et al*., 2000). Microconidia may be

usually borne on simple and short conidiophores, which arise laterally on the hyphae. They are oval to cylindrical, straight or curved and measure 2.5 - 3.5 x 5 – 11 µm. Macroconidia are borne on branched conidiophores, thin-walled, 1 to 6 septate, fusoid, pointed at both ends and measures 3.5 - 4.5 x 25 – 65 µm. Chlamydospores are formed in old cultures, which are smooth or rough walled, terminal intercalary and may be formed singly or in pairs or in chains (singh, 2015).

2.8.2. Life Cycle of the Pathogen

An effective strategy for combating plant diseases requires a thorough the knowledge of the pathogens, including their biology, ecology and their variability. Knowing the life cycle of a pathogen sheds light on its survival mechanism, interaction with host plants, spread over time and space, and capability of evolving into new forms (pathotypes). *Fusarium oxysporum* f.sp. *lentis (Fol)*, lacks a teleomorphic state, and thus genotypic changes result from anamorphic phenomena rather than sexual reproduction that results in teleomorphic stage. The first step in the sexual-like cycle or process is the formation of a heterokaryon, which is important for the wilt fungus to adapt to changing circumstances (Glass and Kuldua, 1992). At this stage, due to the exchange of genetic material among various forms, the pathogenicity partner changes.

Vascular wilt disease caused by fungi is usually highly destructive whether they occur in cultivated crops or in indigenous wild species (Mace *et al*., 1981). Wilts occur as a result of the presence and activities of the pathogen in the xylem vessels of the plant (Agrios, 2005). Nelson *et al*. (1983) reported that some pathogenic forms of *Fusarium* species penetrate the roots directly, whereas others must enter through wounds. Thus, the vascular wounds enhance vascular colonization. The most common sites of direct penetration are located at or near the root tip of both tap root and lateral roots. Following infection of host roots, the fungus crosses the cortex and enters the xylem tissues. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. Entry is either direct through wounds at the point of formation of lateral roots. Direct introduction of the vascular wilt pathogen to the stem does not allow the activation of resistance mechanism present in the roots for soil borne pathogen getting roots as the main point of entry (Cirulli *et al*., 2008). The mycelium takes an intercellular path through the cortex, and enters xylem vessels through the pits. Infection occurs ready where the xylem was exposed by wounding of the stem or the root.

2.8.3. Symptoms of the Pathogen/ Disease symptoms

Lentil Fusarium wilt occurs in fields in patches and originates either at early (seedling) crop stage or at reproductive (adult plant) stage (Chavdarov, 2006). Seedling wilt is characterized by sudden drooping, followed by drying of leaves and seedling death. The roots appear healthy, with reduced proliferation and nodulation and usually no external discoloration of the vascular system. Adult wilt symptoms appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or individual branches (Ahari *et al*., 2011). Wilt symptoms in the field include wilting of older leaves, stunting of plants, shrinking, and curling of leaves from the lower part of the plants that progressively move up to the stems of the infected plant. Plants finally become yellow and die (Pouralibaba *et al*., 2016). Agrois (2005) stated that *Fusarium oxysporum* generally produces symptoms, such as wilting, chlorosis, necrosis, browning of the vascular system, stunting and damping–off.

2.8.4. Ecology and Epidemiology of *F. oxysporum* f.sp. *lentis*

The epidemiology of root-infecting fungi in the soil is complex and factors, such as inoculum density, pathotype, plant age, host resistance and its genetic potential, air and soil temperature, soil moisture, soil nutrients, plant density and other cultural practices, may affect wilt development (Haware and Nene, 1990). Like many other *formae specialis* of *F. oxysporum*, the pathogen (*F. oxysporum* f.sp. *lentis*) has a very limited host range as it only infects lentil in nature. Being soil-borne pathogen of lentil worldwide, it may cause severe damage and complete crop failure under favorable conditions for infection by the pathogen and disease development (Chaudhary and Kaur, 2002).

Fusarium oxysporum sp. is considered as a warm climate pathogen that is generally found in sandy and acidic soils (Agrios, 2005). The optimum temperature for the disease ranges from 20 to 30 °C (Dolatabadi *et al.*, 2011; Mohammadi *et al.*, 2011). Warm soil temperatures ranging from 20 to 30 ºC the most important environmental factors favouring development of the disease (Khare, 1981). It has been reported that wilting was markedly observed during rainy season as well as its spread accelerated with the onset of rainfall and decreased with advancing winter (Prakash and Pandey 2007). Similarly, presence of nematode significantly increases wilt

incidence, causes significant reduction in shoot length, root length and nodulation in both susceptible and resistant varieties (De *et al.,* 2001).

F. oxysporum f.sp. *lentis* is able to survive in the soil for a long period by forming resting spores, thick-walled propagative structures, known as chlamydospores, and dormant mycelium in infected plant debris as well as survive in soil either in dormant form or saprophytically without a suitable host for several years (Taylor *et al*., 2016) and is capable of colonizing residues and roots of most crops grown in rotation with lentil. The causal pathogen is both seed- and soil-borne and can survive in soil, even in the absence of its host for six years (Ayyub *et al*., 2003).

Fusarium wilt is a monocyclic disease and the initial inoculum level is very important for determining the incidence of the disease. Dead roots are suitable substrates for Fusarium survival. However, dead substrates are not as a rule colonized by the fungus if they are already occupied by other fungi, i.e. it implies that the microorganism is ecologically obligate pathogen. The fungus possesses a high competitive saprophytic ability and spreads in the soil, mainly by conidia carried by water movements. The pathogen can survive as hyphae in infected crop debris, as sclerotia in the soil (Hanson, 2005; Porter *et al.,* 2011*),* and thick-walled hyphae can function as chlamydospores (Porter *et al*., 2011). It can also be spread by blowing infested soil or crop debris (Dillard, 2001), and can even be transmitted, to a limited extent, via infected seed (Porter *et al*., 2011). Taylor *et al*. (2016) reported that the fungus can be seed-borne either as systemic infection inside the seeds or as a contaminant on the surface of the seeds.

2.9. Lentil Fusarium Wilt Disease Management

The management of lentil wilt complex can be done through cultural practices, use of resistant varieties, biological control and chemical protection. In the absence of resistant/tolerant varieties, it would be too difficult to manage the disease caused by soil-borne pathogens because of complex soil physico-chemical properties, environmental conditions and biological origin. The following sub-sections deal with possible management options of lentil Fusarium wilt.

2.9.1. Cultural Practices

The cultural practices include deep plowing and leaving the soil fallow. These practices helped in reducing the pathogen population in the soil but do not eliminate it completely (Agrios, 2005).

Also, management practices to reduce the effects of waterlogged soil include genotype choice and the proper design of field drainage systems to discharge excess water (Silvia *et al*., 2016). Selecting cultivars that mature early and adjusting the planting date, if possible, can reduce disease incidence by escaping a portion of lentil growth period from weather conditions favorable to the disease. Use of clean seed for sowing and/or the use of fungicidal seed treatments can eliminate or reduce contaminating inoculum sources. Lentil crops grown on raised beds produced significantly superior agronomic characteristic; yield attributes trials, seed and straw yield as compared to the flat bed sown crop (Rathore *et al.,* 2010). Merkuz and Getachew (2012a) reported that growing resistant and moderately resistant varieties on raised seedbed that drain excess water with recommended seeding rate could reduce plant mortality caused by chickpea wilt.

Changes of micro-environment are complex and often interrelated because they affect both host and root pathogens. Some factors may affect the lentil plant negatively and the fungus positively, leading to an apparent increase in lentil wilt. Under the traditional management systems, lentil yield from Vertisols is far below the potential yield (Berhanu, 1985). Feng *et al.* (2010) also demonstrated significant effects of the ridge-furrow system on Siberian wild rye (*Elymus sibiricus*). Field observation showed that *E. sibiricus* plants in the ridge-furrow system grew more rapidly and robustly than that in flat bed. Salih and Ageeb (1987) showed that sowing date and plant population significantly affected the incidence of wilt and root rot complex.

2.9.2. Host Plant Resistance

Breeding for host resistance is the most effective, economical, efficient and environment-friendly disease management method (Sarwar *et al*., 2014). The search for sources of resistance to diseases is a primary and most eminent research for most of the work carried out in the past and also is continuing presently (Shankar *et al.,* 2013). Successful screening for disease resistance is based on the availability of large and diverse germplasm collections and of precise and accurate screening techniques (Infantino *et al*., 2006). To date host plant resistance screening was being conducted at Debre Zeit International Fusarium wilt by screening of germplasm from abroad and indigenous materials. However, the challenges of lentil Fusarium wilt increased more today than the previous time since the available host plant resistance source was not obtained as a core factor regardless of the breeding efforts made so far.

2.9.3. Biological Control

Biological control relies largely upon an interruption of host parasite relationship through biological means (Snyder, 1960). Biological control implies the control of disease by living microorganisms under their natural or artificial circumstance (Garrett, 1965). Biocontrol is the best and effective substitute, environment friendly, especially against soil-borne pathogens, such as *Fusarium* species (Gohel *et al*., 2007). Among several antagonists used for biological management, *Trichoderma* species are used extensively as biocontrol agents against soil- and seed-borne diseases, such as Fusarium wilt (Etebarian, 2006). These antagonists are saprophytic filamentous fungi, easily growing and produce conidia having long survival period in large quantities (Mohamed and Haggag, 2006). *T. harzianum* was highly efficient in controlling wilt disease and reducing disease severity to 8.9% when applied as a soil drench (Jager *et al*., 1991).

Kumar *et al*. (2013) observed significant reduction in incidence and maximum grain yield in field trials against lentil wilt through *T. harizanum* + *Pseudomonas fluorescens* as bioagents. Likewise, Akrami *et al*. (2011) evaluated three isolates of *Trichoderma*, viz. T1 (*T. harzianum*), T2 (*T. asperellum*) and T3 (*T. virens*) alone and in combination against lentil Fusarium wilt.

2.9.4. Chemical Control

Under high inoculum conditions, suitable seed dressing fungicides are also most effective besides biocontrol agents (Garkoti *et al*., 2013). Presence of high mutations and variations among the pathogen populations limit the effectiveness of natural resistance in the host plants against the pathogens (Nimbalkar *et al*., 2006). Shukla *et al.* (1972) found that seed treatment with brassicol 0.2% was effective against root rot of lentil caused by *Rhizoctonia solani* under field conditions. Singh *et al.* (2014) studied the efficacy of different treatment measures, *viz*. carbendazim, benomyl and vitavax against seedborne fungi, such as *Alternaria alternata, Aspergillus, Fusarium, Penicillium* and *Rhizopus* spp.

2.9.5. Integrated Disease Management

Integrated disease management is a holistic approach that combines available disease management technologies in an economically and ecologically-sound manner (Agrawal *et al.*, 2002). Landa *et al.* (2004) studied the effect of sowing date, resistant genotypes and seed and

soil treatments chemically or biologically against Fusarium wilt and found it effective against wilt incidence. Blanca *et al*. (2004) reported that the change in date of sowing, host plant resistance and seed/soil treatment with biocontrol agents reduces disease intensity and increases lentil seedling emergence. Thus, an integrated disease management approach is essential to combat lentil Fusarium wilt for increased and sustainable yields.

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

The study consisted of two separate components, namely lentil Fusarium wilt survey, to assess the prevalence, distribution, and incidence lentil wilt as well as field experiment, to determine the effect of varieties in combination with different seedbed types on Fusarium wilt incidence and lentil yield parameters.

3.1.1. Survey Areas and Disease Assessments

Two administrative zones in Amhara (North Shewa) and Oromia (East Shoa) regional states located in the central highlands known for large lentil area coverage were selected for disease survey. Three districts from East Shewa Zone (Ada'a, Lume, Gimbichu) and five districts from North Shewa Zone (Minjar Shenkora, Siyadebrena wayu, Moretina-jiru, Aleltu and Ensaro) were surveyed (Figure 1). The survey was conducted at obtained different growth stages (from seedling to pod setting) of the lentil crop. The survey was conducted twice including lentil crop planted in July up to August and from end of September up to early October during 2017 cropping season. Using stratified sampling procedure, farmers' fields were randomely selected from each kebele. Formal survey questionnaires were prepared to capture data from farmers via personal interviews. A total of eight districts and consisting a total of 24 PAs were purposively selected for their high lentil production potential areas and road accessibility. A total of 192 farmers' fields were surveyed in eight districts of central highlands of Ethiopia.

In each field, disease samples were selected and collected from five equally spaced areas in a diagonal ''X" fashion with $0.25m^{2}$ quadrat. Symptomatic plants showing wilting, yellowing of the leaves (chlorosis), vascular discoloration (browning of the vascular system, i.e. xylem vessels but not always), dull green, suddenly dropping of older leaves and death of leaves were recorded. From each field, about 25 infected lentil plants were uprooted and specimens were kept in paper bags for isolation in the laboratory to determine associated of symptomatic plants with the wilt pathogen. The percentage of plants showing disease symptoms in each quadrat was recorded and average of the five quadrats were calculated for each field as a disease incidence.

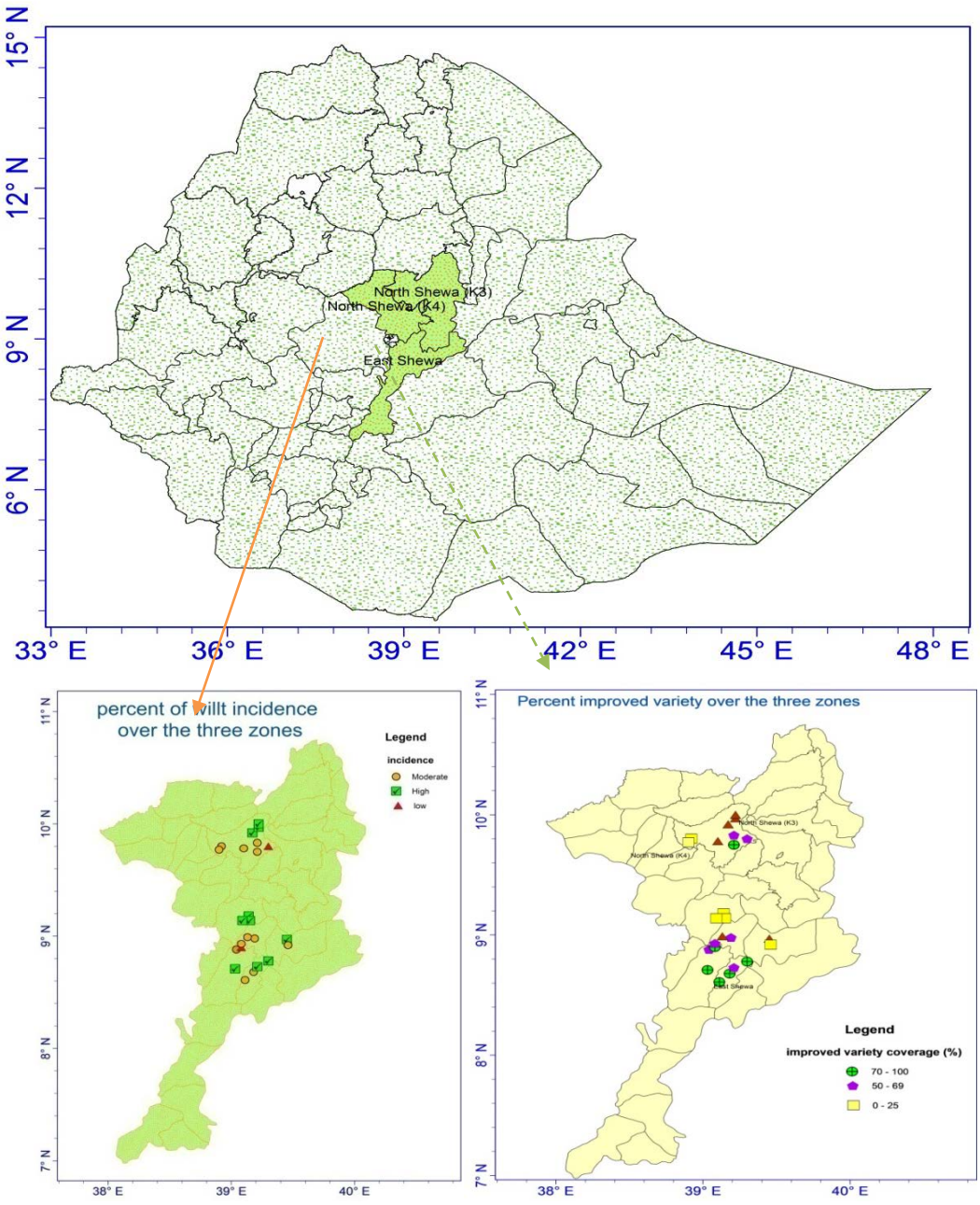


Figure 11. Map showing disease survey locations in eight districts of central highlands of Ethiopia

A global positioning system (GPS) was used to determine the coordinates (latitude and longitude) and altitude of each field visited during field inspections. During the survey, all important variables like field history, field size, crop variety, cropping systems (sole or intercropping), date of planting (early or late), weeding practice, application of fertilizer and

rate, planting method, soil types, crop growth stage, seed rate and previous crop were recorded to observe their relationship with disease incidence.

Both disease prevalence and incidence were calculated using the following formula.

$$\text{Disease prevalence (\%)} = \frac{\text{No of fields showing lentil wilt}}{\text{Total number of fields visited}} \times 100$$

Percent disease incidence was calculated using the following formula:

$$\text{Wilt incidence (\%)} = \frac{\text{Wilted plants in quadrat}}{\text{Total number of plants assessed in quadrat}} \times 100$$

3.1.2. Pathogen Isolation

Potato Dextrose Agar (PDA), a general purpose medium, was used for isolation of the pathogens associated with diseased lentil plant samples. One hundred ninety-two representative diseased lentil samples were used to identify the pathogens associated with infected lentil plant specimens. Infected root and stem samples were cut into ~1 cm pieces using sterile scissors and washed under running tap water. The segments were surface sterilized with 1.5% sodium hypochlorite solution for 2-3 min, washed three times with sterile distilled water, and blotted on filter papers and five sections of the dried parts were aseptically placed on potato dextrose agar (PDA) containing chloramphenicol (0.05 g L^{-1}) as bacteriostatic agent and poured in 9 cm petri dishes and incubated at 25 °C for 7 days (Lee *et al*., 2007). Sub-cultures were later prepared to get pure cultures. Slides were prepared from these pure cultures and examined microscopically and identification was made using key (Nelson *et al*., 1983). Colonies were sub-cultured on fresh PDA plates using a hyphal tip technique to obtain pure cultures. The isolates were subsequently identified according to their cultural and morphological features (Leslie and Summerell, 2006).

3.1.3. Pathogenicity Assay of *Fusarium* spp.

Pathogenicity test of the representative *Fusarium oxysporum* fungus isolated from wilted specimens of lentil was evaluated to determine whether or not the *Fusarium oxysporum* isolates were pathogenic to lentil using a susceptible check, ILL-590. *Fusarium oxysporium* isolates, which were pathogenic to susceptible lentil, were separated from symptomatic lentil plant specimens in the study area. Purified *Fusarium oxysporum* isolates cultures were used to produce inoculum. Prepared sand-maize meal medium and 20 ml of distilled water in each 250 ml beaker was autoclaved at 15 psi for 20 minutes.

For mass multiplication of the inoculum, each sterilized beaker was inoculated with 7 mm disk of actively-growing fungal culture and was incubated at 25 °C for 15 days. Maize substrate is known to be efficient for mass production of inoculum for pathogenicity assay (ICRISAT, 2011). Pots were washed thoroughly with distilled sterilized water, dried and then sprayed with spirit to avoid saprophytic contamination. All plastic pots (15 cm) were filled with sterilized soil (2 kg pot^{-1}) and inoculated (Dubey *et al*., 2012). Two pots were used as replicates for each isolate using CRD. The sterilized soil in each pot was mixed with maize grains free of any fungi. Ten lentil seeds (surface-sterilized with 1.5% sodium hypochlorite for 2 min) of susceptible lentil genotype was sown in each pot. Adequate and regular watering was performed as necessary.

3.2. Survey of Lentil Seed to Determine its Infection Level by Fol

Seed samples were collected from different farmers' fields, open markets and research centers in central highlands of Ethiopia. A total of 100 lentil seed samples (500 g seed per sample) consisting 30 samples from each farmers' saved and an open market, and 40 samples from Debre Zeit and Debre-birhan research centers were collected. Hundred lentil seeds were randomly taken from each sample, and surface-disinfected by soaking in 1.5% sodium hypochlorite (NaOCl) solution for 2 min with constant agitation. Surface-disinfected seeds were rinsed with sterile distilled water for one minute. Seed infection level was conducted by using agar plate method. Seeds were placed at the rate of 15 seeds per petri plate (15cm) containing 30 ml of potato dextrose agar (PDA) and incubated at 25 °C for 7 days. After seven days of incubation, the fungal colony growth was examined under compound microscope (Khare, 1996).

3.3. Effect of Varieties and Seedbed Types Integration on Fusarium Wilt and Lentil Yield

Field experiments were conducted in field with history of naturally infested with wilt/root rot causing pathogens at Debre Zeit and Chefe Donsa Research sites in 2017/18 cropping season. Debre Zeit is located at latitude 08º44′N, longitude 38º58′E, an altitude of 1900 m.a.s.l. and received average annual rainfall of about 851 mm and the mean annual and minimum temperatures of 28.3 and 8.9 ^{o}C, respectively. Chefe Donsa is located at latitude 08º57′N, longitude 39º06′'E, altitude of 2450 m.a.s.l. and received average annual rainfall of about 900 mm and the mean annual and minimum temperatures of 26.0 and 7.0 ^{o}C. Both sites have vertisol with waterlogging problem.

3.3.1. Experimental Materials and Procedures

Four lentil genotypes (ILL-590, Alemaya, Denbi and Derash) with varying levels of resistance and four seedbed types (flat, open raised, farmer's practice and tie-ridge seedbed) were used in the study. The lentil genotypes, Alemaya, Derash and Denbi are moderately resistant and ILL-590 (Susceptible check). The experiment was laid out in split plot design (seedbed types as main plot and lentil genotypes as sub-plot) with three replications. The plot size was 3.2 m^2 (4 rows per plot); with row length of 4 m. The seed rate was 800 seeds per plot. Lentil seeds were drilled by hand at a depth of 3-3.5 cm.

3.3.2. Data Collected

Lentil Fusarium wilt might occur at any growth stage from seedling to the reproductive stages. Thus, visual observations of disease symptoms was started a two weeks after emergence and total number of wilt infected plants were recorded till the crop approached near physiological maturity in each lentil varietal plot for each population to calculate disease incidence by using the formula presented below.

3.3.2.1. Disease Incidence Assessment

Disease incidence was recorded four times at every fifteen days starting from the first appearance of disease symptoms. Plants that showed complete or partial wilting were considered as wilted and staked to avoid double counting in subsequent assessments. Percent of wilt incidence was calculated on the basis of initial plant count and total number of diseases plants in each plot using the following formula (Chavdarov, 2006).

$$\text{Disease incidence (\%)} = \frac{\text{Total number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

3.3.2.2. Area Under Disease Progress Curve

Area under disease progress curve (AUDPC) value was used in the analysis of variance to compare amount of disease among plots with different treatments. Area under the disease progress curve (AUDPC) and rate of disease development were calculated to evaluate treatement effects in the experiments at both locations. The area under the disease progress

curve (AUDPC) was computed from disease incidence for each plot as described by Campbell and Madden (1990):

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

Where,

n = Total number assessment times,

ti = Time of the i^{th} assessment in days from the first assessment date,

xi = Percentage of disease incidence at i^{th} assessment.

Where, xi = is the cumulative disease incidence expressed as a proportion at the i^{th} observation (percent disease incidence),

ti = is the time (days after sowing) at the i^{th} observation and n is total number of observations.

3.3.3. Agronomic Data

Emergence Count: The total plant population was counted from two central rows after emergence.

Days to 50% flowering: Days from sowing to when 50% of the plants per plot started flowering.

Days to physiological maturity: Days from sowing to when 90% of the plants per plot reached maturity.

Biomass/biological yield (BY) (kg ha^{-1}): This was determined as the weight in kilogram of aboveground parts of the plants. At maturity, the whole aboveground parts of plants, including leaves, stems, and seeds from the net plot area were harvested and the biomass was measured or weighed with spring balance.

Grain Yield (kg ha^{-1}): Grain yield (GY) was determined from the harvest of the net plots. The sample was harvested from the net area of each plot and then threshed and cleaned. After cleaning, the grain weight was adjusted to moisture (assuming 10.5%, wet basis) and was weighed by sensitive balance. Yield harvested from the two middle rows was converted into kilogram per hectare (kg ha^{-1}).

$$\text{Gy= (kg ha}^{-1}\text{)} = \frac{\text{Grain yield obtained (kg /ha) X}(100-\%\text{ moisture content})}{(100-10.5)}$$

3.4. Statistical Data Analysis

Analysis of variance (ANOVA) was performed for disease parameters, growth and yield parameters and subjected to analysis of variance (ANOVA). ANOVA was performed using general linear model (GLM) of the statistical analysis system`s procedure (SAS, 2002). Least significance difference was used to separate treatment means. Correlation coefficients were also calculated to explain the degree of relationship between parameters. Disease progress rate was analysed using the statistical software called Minitab, version or Release 15.0 for windows®, 2007. The F- statistic was used to test the significance of variance ratios (comparision between two variances). The coefficient of determination (R^2) was computed to determine the proportion of the variation in management practices explained by the disease variables (predictors) in the model. To determine the disease progress rate from the linear regression, a monomolecular model, ln[(1/1-y)] was used to estimate the disease progress from each separate treatment (Campbell and Madden, 1990).

4. RESULTS AND DISCUSSION

4.1. Distribution and Relative Importance of Lentil Fusarium Wilt

Lentil wilt caused by *Fusarium oxysporum f. sp. lentis* was the most frequently occurring and devastating disease in the surveyed districts. The disease was 100% prevalent in all the surveyed districts, during 2017 main cropping season (Tables 1 and 2). However, the level of wilt incidence was varied from 2% in Lume up to 75 in Moretina-Jiru farmers' fields. Dubey *et al*. (2010) surveyed different states of India and found Fusarium wilt incidence of chickpea varied from 14.1 to 32.0 %, also said that it is prevalent in almost all chickpea growing areas of the world. Percent incidence was equal to stand loss (%) because of the fact that the infected plants will not recover and diseased plants are not used by the community for any purpose.

Table 1. Mean percent wilt incidence in villages of studied districts at early lentil planted

District	Village name	No. of fields per village	prevalence %	Wilt incidence for village (%)	Mean wilt incidence per district (%)
Adaa	Kataba	10	100	11.0	
	Akako	7	100	9.0	14.5
	Dankaka	7	100	22.14	
Gimbichu	H/seftu	9	100	13.22	
	Uso	8	100	11.88	14.6
	Girmi	7	100	15.71	
Lume	Tullu re'e	10	100	10.0	
	Danse	8	100	14.0	20.2
	Nannawa	6	100	25.0	
Minjar-Shenkora	Zewelde	6	100	20.0	
	Korma	8	100	10.0	18.1
	Chale	9	100	17.	

The research results demonstrated that high mean disease incidence observed in Muratina-Jiru (32.8%), followed by Aleltu (28.0%) (Table 2). However, the lowest (13.2%) mean incidence of lentil wilt was recorded in Siyadebrena Wayu, followed by Adaa (14.5%) and Gimbichu (14.6%) districts (Tables 1 and 2). In a similar study, Chaudhary *et al*. (2010) recorded a range of 0.7 - 9.3% mean plant mortality and an overall mean mortality of 6.3% due to lentil wilt incidence in 116 lentil-growing districts of India. Likewise, Hamdi and Hassane in (1996) reported low incidence of lentil wilt (5% to 10%) in Egypt. Bayaa *et al*. (1986) reported 2% to 70% wilted

plants with a mean of 12% in 27 fields in Syria. Previously, Tadesse *et al*. (1999a) reported an average of 10% plant mortality in 32 lentil fields; but later, Bekele *et al*. (2005) recorded an average of 52% plant infection in 8 lentil fields. Similarly, high levels of plant infection 70% in lentil fields were reported in Syria (Makkouk *et al*., 1992) and Pakistan (Makkouk *et al*., 2001).

Table 2. Percent occurrence of wilted lentil plants in villages of studied districts at late planted lentil in 2017 main cropping season

District	Village name	No. of farmer per village	Prevalence (%)	Wilt for village incidence (%)	Mean of incidence (%) per district.
Siyadebrena-Wayu	Ejersa	7	100	15.0	13.2
	D/kombolcha	9	100	13.0	
	Jihur	9	100	7.6	
Moretina-Jiru	Bolo	7	100	29.3	32.8
	weranba	8	100	32.5	
	Agegn	9	100	22.2	
Ensaro	Wakelo	7	100	21.5	16.0
	Diramu	9	100	12.6	
	Denbi	8	100	14.8	
Aleltu	Tokuye	10	100	32.5	28.0
	Wegdera	6	100	27.5	
	Wera	8	100	23.8	

The lower wilt incidence in Gimbichu district as compared to all other surveyed districts except Siyadeberena-wayu, and Adaa might be explained by the nature of lentil cultivars coupled with the farmers' seedbed (*shurube*) preparation type to produce lentil in the district. Similarly, in Siyadebrena-wayu where the disease incidence is low, farmers plant lentil on a good drained seedbed (*shurub*e) irrespective of cultivars. The most dominant local varieties were observed in Aleltu (91.7%), Ensaro (83.3%, Moretina jiru (62.5%) and Minjar-shenkora (62.5%), in their order while the improved varieties were available in Lume (83.3%), Adaa (75%), Siyadebrena wayu (58.3%) and Gimbichu (54.2%) districts. This indicated that there were no improved varieties available for most of the producers. Similarly, earlier studies by Tessema (2006) showed that in the central highlands of Ethiopia adoption of improved lentil varieties was found to be below expectations and only 9% of the lentil growers adopted improved varieties.

Fusarium wilt was associated with lentil varieties at all dates of sowing in both early and late planted as well as at all growing stages of the crop. However, numerically higher level of the

pathogen association was observed in late lentil planted than in early planting season (Tables 1 and 2) and variation in wilt incidence among the surveyed districts might be due to variation in environmental factors and type of management options used by individual farmers. This is in agreement with the finding of Nighat *et al*. (2012) who found that the percentage of wilted plants ranged from 0 to 32.9% in the early season wilt and from 9.60 to 100% in the late season wilt. The higher disease incidence in late sown crop might be due to high temperature that prevailing the development of pathogen. This observation is consistent with the earlier findings of Aziz (1992) who reported delay in sowing causes reduction in lentil yield. Not surprisingly, if the crop is planted too late, farmers risk crop yield loss due to terminal drought when residual soil moisture is depleted. Similarly, Turk *et al*. (2003) reported that the number of pods per plant decreased with delay in planting.

The survey showed that wilt damage takes place at any planting dates under favorable conditions however late sowing of the lentil was severely aggraved lentil wilt might be increased the temperature agreed with (Mustafa, 2009) reported as mainly attack whenever suitable conditions (high temperature). Environmental conditions, such as high soil moisture, high soil temperature and continuous cropping also contribute to increased incidence of wilts and root rots (Doohan *et al*., 2003). Same results were also reported by Navas-cortes *et al*. (2001) who indicated that incidence of wilt was positively correlated with increasing soil temperature and inoculum density of the pathogen. Temperature and soil moisture are the main climatic and edaphic factors in determining fungal growth rates and wilt symptom expression (Falahati *et al.*, 2010).

The current observations showed higher disease incidence in poor soil drainage was observed. Water-logging contributes to the dieback of the primary root system of legume species (Deckers *et al.,* 2001); and the period subsequent to submersion is not sufficient for a full recovery of the roots and this might aggravate wilt and root rots and decreases the stomatal conductance or leaf water potential and also affects the processes associated with solute movement across membranes, such as nutrients uptake. Similarly, Barrett-Lennard *et al*. (1988a) reported that the initial growth of roots and shoots rapidly decreased in waterlogged soil conditions. Crop production in waterlogged soil greatly decreased as compared to the non-waterlogged soil. This observation was well supported by Silvia *et al*. (2016) who reported as management practices to reduce the effects of waterlogged soil and included use of proper design of field drainage

systems to discharge excess water or soil moisture and choice of varieties. Bhatti and Kraft (1992) reported that soil moisture plays a key role in wilt and root rot development in chickpea. Similarly, faba bean root rot was associated with waterlogged soil, vertisols in the highlands of Ethiopia (Belete *et al.*, 2013).

4.2. Influence of wilt Disease with Biophysical Factors

Significant differences in wilt disease incidence were observed among lentil genotypes, growth stages, land preparation methods, fertilizer application, ploughing frequency and drainage soil moisture while weeding practices, soil types, previous crop, seed rate, and planting date did not showed statisticlly significant differences associated with wilt incidence (Appendix Table 5).

Table 3. Effect of lentil cultivars, growth stages, seedbed types, fertilizer application and drainage on wilt incidence % during the 2018 cropping season

Variables	Number of fields with respective variable	Mean disease incidence (%)
Lentil cultivars		
Improved	85	13.0
Local	107	17.8
Lentil growth stages		
Seedling	14	10.6
Vegetative	81	16.3
Flowering/reproductive	79	18.0
Podding	18	15.5
Seedbed types		
Flat	131	16.6
local shurube	61	13.5
Fertilizer application		
DAP	29	13.8
UREA + DAP	42	12.8
NONE	121	18.5
Soil drainage		
Good	35	11.4
Moderate	115	17.7
Poor	40	20.0
Very good	2	11.0

Mean wilt incidence varied from one district to another and from one farm to another as affected by different biophysical factors. Survey data showed that wilt damage takes place at any crop

stage of lentil under favorable conditions. Lentil crops at the seedling stage showed slightly lower disease incidence (10.6%) than those at the vegetative (16.3%) and high Fusarium wilt incidence was observed at flowering (18.0%) stage (Table 3). This finding of which is in agreement with the observation by Shakoor (1991) reported that the disease occurs at seedling as well as at flowering and pod setting stages with more incidence at flowering and podding stages when there is high temperature (25°C) and moisture stress prevails.

Similarly, Chaudhary *et al*. (2006) reported that the wilt incidence during reproductive growth was correlated with yield loss estimates, with a reduction in seed yield per unit change in wilt incidence and high association of *F. oxysporum* (66.3%) as compared to other fungi with lentil. In Pakistan, heavy plant mortality of lentil (may cause 10-50% crop loss every year) due to wilt at different growth stages of the crop was reported by Chaudhary *et al*. (2009).

Higher wilt incidence was computed where the crop was planted in the poor drainage soil than others likely reflecting the wet conditions that favor disease development. The amount of moisture retained in the soil is a function of soil type (Yanar *et al*., 1997). The types of soil present in a farmer's field may or may not predispose a crop to severe disease outbreaks. Indeed, we observed a correlation between soil moisture and disease incidence, with fields containing poor drainage soil also experiencing the highest incidence of wilt root rot (Table 3). The vertisol soil type is known to retain more moisture than the lighter soils found in other sites (Geletu *et al*., 1994), thus favoring wilt root rot pathogens (Bhatti and Kraft, 1992). Similarly, high incidence of wilt in chickpea crops grown in black soil has previously been reported by other researchers (Rachana, 2002). Broad bed and furrow (BBF) was recommended as a means to overcome the problem of poor drainage to enhance productivity of crops sensitive to waterlogged soil in the central highlands of the country (Teklu *et al.,* 2006).

Data from the present study indicated that local varieties or landraces were susceptible to Fusarum wilt; and were predominantly grown by the smallholder farmers in all the surveyed districts. The key biophysical factors affecting wilt epidemic was the use of highly susceptible lentil landraces on poor drainage soil moisture. The present observation showed that the local variety favored Fusarium wilt incidence and revealed the presence of the pathogen at high levels in all lentil-growing areas (Table 3). Previous studies indicated that lentil productivity, particularly in Ethiopia, remains low due mainly to cultivation of low yielding and disease

susceptible landraces (Asnake and Geletu, 2004). Ghosh *et al*. (2013) noted same problem in India and reported that local cultivars had a higher incidence of lentil wilt. This was a major indicator of how much the yield of lentil crop is low due to use of local varieties and, hence, advisable to use improved varieties because improved varieties can yield 1.4-5.0 t ha^{-1} under research fields and 0.9-3.0 t ha^{-1} under farmers' fields with full use of recommended agronomic packages (Abraham, 2015).

4.3. Isolation, Purification and Identification of Pathogens Associated with Lentil Wilt

4.3.1. Culturing and morphological characterization of pathogens associated with lentil

Identification of colony cultures revealed that *Fusarium oxysporum* was the most abundant (100% in frequency) fungus found in association with Fusarium wilt infected samples (Table 4). The fungus was isolated from nearly 100% of the samples (Table 4).

Table 4. Prevalence and average fungal colony formed from infected lentil plant across the eight surveyed districts in central highlands of Ethiopia during 2017 main cropping season.

Districts	**Total colony formed (%) of isolated fungi from infected lentil wilt**					
	Fusarium oxysporum spp.		*Rhizoctonia* spp.		*Sclerotium* spp.	
	Prevalence (%)	Average colony formed	Prevalence (%)	Average colony formed	Prevalence (%)	Average colony formed
Ada'a	100	62.2	33.3	7.8	8.3	2.2
Aleltu	100	56.3	12.5	15.0	0	0.0
Ensaro	100	66.4	0	0.0	0	0.0
Gimbichu	100	51.5	29.2	4.7	4.2	1.1
Lume	100	57.8	70.8	21.7	0	0
M/jiru	100	68.3	0	0.0	0	0.0
Minjar	100	50.4	41.7	10.7	4.2	1.1
S/wayu	100	63.2	0	0	4.2	1.1
Mean	100	60.0	23.4	7.5	3.0	0.7

Initially the fungus produced white mycelial growth turning light buff or deep brown later and fluffy or submerged on dextrose agar (PDA) at 25 ^{o}C and had septation and profusely branched growth. Then its growth became felted or wrinkled as well as sporulated in old cultures. The second most frequent fungus(i) in the cultures were *Rhizoctonia* spp. (23.4%) and *Sclerotium* rolfsii was the least (3.0%) frequent fungus identified in the cultures (Table 4). Similar trends have been reported in Uttar Pradesh, India where *Fusarium oxysporum* f. sp. *lentis* and

Rhizoctonia bataticola were isolated from 43.8% and 25.7% of lentil samples with wilt complex respectively (Chaudhary *et al*., 2006). These findings indicate that Fusarium wilt was the most important soilborne disease of lentil in the central highlands of Ethiopia. Similarly, Narasimhan (1929) observed association of *Rhizoctonia* spp. and *Fusarium* spp. with wilted chickpea plants.

In this study, *Fusarium oxysporum* exhibited variations in colony characteristics, such as color and shape. Colony characteristics of the isolates showed fluffy growth pattern with white to pinkish white mycelial color. This was supported by Leslie and Summerell (2006) reported that hyphae pale brown or brown, branched, with nearly right-angled side branches constricted basally, septated closely between main hyphae and side branches.

The most dominant colony colors were primarily cotton like whitish, purple, pinkish and deep brown were observed in the culture on solid medium. In past studies various types of pigmentations (yellow, brown, whitish) in culture has been recorded (Saxena and Singh, 1987). Conidia shapes were curved, slightly curved and oval as well as pointed at both ends like that of banana. The study showed that three septate and zero to one septate conidia were commonly observed in both macro- and micro-conidia, respectively. Chlamydospores were formed singly and/or in pairs. The current findings in agreement with the observation by Dubey *et al*. (2010).

4.3.2. Colony Reverse Morphology

Dark blue colors or yellow/orange or dark purple were observed on the undersurface of PDA plate. This finding is consistent with that of Kontoyiannis *et al*. (2000) who commented that the colors of the colony may be white, cream, yellow, red, violet, pink or purple and on the reverse, it may be colorless, tan, red, dark purple, or brown. Then the hyphae had pale-brown or brown, branched, with nearly right-angled side branches constricted basally at old colonies. A similar observation was made by Sneh *et al.* (1996) who reported in older colonies the hyphae often branch at right angles and at any place along the cell. Similarly, they observed that the sclerotic color ranged from brown to dark-brown with various shapes.

4.3.4. Pathogenicity Assay

All the seventy two (three from each district) representative isolates of *F. oxysporum* collected from the surveyed areas were pathogenic to the susceptible lentil genotype, ILL-590. The plant

mortality was varied from 28.6 upt to 80.0%. Similarly the level of virulence (82%) in *F. oxysporum* f.sp. *lentis* isolates was reported by Taheri *et al*. (2010). The symptom produced on lentil in pot experiment was identical to those observed in the field and yellowing of the leaves, suddenly dropping of old leaves, curling, narrowing the leaf area and dead plant was observed.

Typical lentil Fusarium wilt symptom appeared three weeks after fungus inoculation into sterile soil for some very aggressive isolates. Cultures derived from re-isolation were morphologically identical to the parent culture. There was no symptom development on the lentil plants in the untreated control pots. As re-isolation studies confirm the presence of the same fungus identical to the original one obtained from naturally wilted plants, the *F. oxysporum* found in association with wilted lentil plants collected from the central highlands of Ethiopia is the most problematic pathogen of lentil wilt. Identification of the causal agent, *F. oxysporum* to the forma specialis (f.sp.) level, i.e. f.sp. *lentis*, though, requires further study using conventional and modern (molecular) techniques.

4.4. Lentil Seed Healthy Test

Among the seed samples studied the fungi, *Fusarium oxysporum* spp., *Alternaria* spp., *Aspergillus* spp., *Rhizoctonia* spp., and *Penicillium* spp., were detected and was associated with lentil seed (Table 5).

Table 5. Fungi associated with lentil seed samples collected from markets, farmers and research centers

Isolated fungi	Ocuurance of frequency (%) isolated fungi from different sources		
	Research center	Farmer saved	Open market
F. oxysporum spp.	2.33 %	3.33 %	4.1 %
Alternaria spp.	0.06 %	0.81 %	1.26 %
Aspergillus spp.	0.78 %	0.37 %	0.59 %
Penicillium spp.	0.83 %	1.7 %	1.33 %
Rhizoctonia spp.	0.83 %	0.67 %	0.37 %

The seed sample collected from Research Center was less infected than seed sample from both farmers' saved and open markets. The highest (15.6%) seed infection level was recorded from seed sample code 033, followed by seed sample codes 002 (13.3%) and 012 (13.3%) from Research Center (Table 6).

Table 6. Detection of lentil Fusarium wilt seed infection level test in 2017 main cropping season

Number	Research source		Open market		Farmer store	
	Sample code	SIL[1] (%)	Sample code	SIL[1] (%)	Sample code	SIL[1] (%)
1	001	4.4	041	8.9	071	0.0
2	002	13.3	042	4.4	072	0.0
3	003	6.7	043	2.2	073	4.4
4	004	8.9	044	4.4	074	0.0
5	005	6.7	045	2.2	075	2.2
6	006	0.0	046	6.7	076	4.4
7	007	0.0	047	0.0	077	0.0
8	008	0.0	048	22.2	078	0.0
9	009	0.0	049	0.0	079	0.0
10	010	4.4	050	0.0	080	0.0
11	011	2.2	051	0.0	081	2.2
12	012	13.3	052	0.0	082	4.4
13	013	0.0	053	4.4	083	8.9
14	014	0.0	054	2.2	084	2.2
15	015	0.0	055	0.0	085	0.0
16	016	0.0	056	2.2	086	4.4
17	017	0.0	057	20.0	087	4.4
18	018	0.0	058	0.0	088	4.4
19	019	0.0	059	0.0	089	0.0
20	020	0.0	060	2.2	090	2.2
21	021	0.0	061	0.0	091	2.2
22	022	0.0	062	2.2	092	0.0
23	023	0.0	063	0.0	093	0.0
24	024	0.0	064	0.0	094	11.1
25	025	0.0	065	2.2	095	8.9
26	026	0.0	066	0.0	096	8.9
27	027	0.0	067	0.0	097	4.4
28	028	8.9	068	4.4	098	4.4
29	029	0.0	069	0.0	099	20.0
30	030	0.0	070	2.2	100	18.0
31	031	0.0	---	---	---	---
32	032	0.0	---	---	---	---
33	033	15.6	---	---	---	---
34	034	0.0	---	---	---	---
35	035	0.0	---	---	---	---
36	036	0.0	---	---	---	---
37	037	0.0	---	---	---	---
38	038	0.0	---	---	---	---
39	039	6.7	---	---	---	---
40	040	2.2	---	---	---	---

[1] SIL= Seed infection level (%).

The results showed that the seed infection level ranged from 0 to 22.2% on seed samples collected from central highlands of Ethiopia during 2017 cropping season. *Fusarium oxysporum* had higher seed infection level than others fungi. The highest (22.2%) seed infection level was recorded on seed sample code 048 collected from open market, followed by seed sample code 057 (20.0%) (Table 6). The lowest (0%) seed infection level due to fungi was recorded on about 50% seed samples (Table 6). Similarly, Singh (2013) evaluated lentil seed for resistance against Fusarium wilt and reported 14 seeds as totally free from infection. The association of pathogens with lentil seed sampled was more serious from the open market sampled might be due to different mixture of the seeds. Simirarly, Karim (2005) reported that seed infection level was highest on seed sample from the open market as compared to farmers' seed sample. The study indicated that *Fusarium oxysporum* was one of the transimitted source of inoculum with limited extent via the infected lentil seeds. The current finding is in agreement with the investigations by (Porter *et al*. (2011) and Taylor *et al*. (2016) who reported that the fungus can be seedborne as systemic infection inside the seeds. Similarly, Singh (2015) reported that lentil is attacked by a number of seed- and soil-borne pathogens.

While for seed samples collected from farmers' saved, the highest seed incidence level was found to be on sample code 099 (20.0%), followed by seed sample code 100 (18.0%) (Table 6). The study indicated that lentil Fusarium wilt can be transmitted through seeds with a limited extent and the finding was in agreement with the observation by Haware *et al*. (1990) who found the infection level of lentil by Fusarium wilt as very low. In Ethiopia, Abraham and Makkouk (2002) examined 270 lentil seed lots and found PSbMV seed transmission in 31% of the lots, with up to 17% transmission levels. Similarly, Ayyub *et al.* (2003) described from Pakistan that the disease is soil and seed-borne. In case of lentil, Kumar *et al*. (2002) detected seedborne fungi from four varieties of lentil and isolated six different fungal genera in each variety.

This indicated that the lentil seed might be a transmission way of Fusarium wilt pathogen and carefully healthy seed free from pathogen will be used. Embaby and Abdel-Galil (2006) found that Fusarium was the common species isolated from some legume (bean, cowpea and lupine), emerging in 5.6%, 4.4% and 4.4% of total fungi, respectively and the most common species was *F. oxysporum*. Thus, the movement of seeds in the open markets allows movement of the pathogen via seed and contributes to spread of disease.

4.5. Effects of Seedbed and Lentil Genotypes on Disease Parameters and Yield

4.5.1. Effects of varieties and seedbed types on lentil plant count after emergence

The results of the analysis of variance (ANOVA) indicated that all the three effects [the two main effects (effects of seedbed types and varieties)] and the interaction effect were non-significant ($P \leq 0.05$) at Debre Zeit (Appendix Table 3). Perhaps, the non-significant main and interaction effects observed at Debre Zeit might be due to the occurrence of Fusarium wilt infection after stand establishment (about 3 weeks after planting) and the probable onset and progress of the disease (wilt) thereafter on the microclimate conditions. However, all the three effects [the two main effects (effects of seedbed types and varieties)] and the inter action effect were significant ($P \leq 0.05$) at Chefe Donsa (Appendix Table 4). On the other hand, the poor vegetative performance of lentils under no drainage conditions is related to water logging, which caused poor aeration of the roots and poor nutrient uptake leading to weak growth and development and hence low crop yield. The result is consistent with the previous findings, a better growth and yield of legumes like lentils under raised bed due to enhanced drainage was reported (Abate *et al*., 1993). Stand count of lentil plant at emergence was used to calculate the disease incidence percent through counting the wilted plants from the whole population per two central rows starting from the first wilt symptom appeared.

4.5.2. Effects of Lentil varieties and seedbed types on lentil Fusarium wilt

ANOVA result showed significant ($P \leq 0.05$) difference among the seedbed types and varieties in the LFWI throughout the epidemic period at all assessment dates (Appendix Table 1). The first lentil Fusarium wilt symptom appeared at 25 days after planting (DAP). The performance of variety was play great role against to fungus than seedbed. This finding is similar to the observation by Taylor *et al*. (2007) and Pouralibaba *et al*. (2015) who found that the use of resistant varieties is the most effective, economical and environment-friendly method to manage lentil Fusarium wilt. On the other hand, Teklu (2006) reported previously, in which seedbed type induced the highest surface runoff as compared to farmers' practice and flat seedbed for vertisols in the central highlands of Ethiopia. This result was in accordance with the investigations of Cowie *et al*. (1996a) who observed that persistence of wetness within rooting zone adversely

affect the crop growth since legumes are too sensitive to high soil moisture. Thus, raised bed was known to reduce the incidence of wilt (Tripathi *et al*., 2013).

Interaction effects of lentil varieties and seedbed types with respect to disease incidence (%) showed significant ($P \leq 0.05$) difference at all wilt recording time starting from the first onset of Fusarium wilt incidence at both locations (Appendix Table 1).

The highest (82.2% and 67.5%) final Fusarium wilt incidence was obtained by planting susceptible check, ILL-590 on flat seedbed and the lowest 25.14% and 8.8% final wilt incidence was obtained from the integration of cv. Derash with raised seedbed at Debre Zeit and Chefe Donsa, respectively (Tables 7 and 8). This might be excess soil moisture condition predisposes resistant varieties to be easily attacked by pathogens, which are not problems during normal growing seasons and facilitating spore germination and penetration into the host by the pathogen. Similar results were reported by Isleib (2014) and Binagwa *et al*. (2016) who also observed the high population of Fusarium wilt fungus that can also be explained by presence of high soil moisture, poor drainage of excess soil moisture and soil compaction that favour the pathogen development.

Table 7. Interaction effects of lentil variety and seedbed type on Fusarium wilt (*F.oxysporium* f.sp. *lentis*) of final disease incidence (%) at Debre Zeit, Ethiopia, in 2017 main cropping season

Treatment combinations	Final percent of disease incidence at every 15 days interval at Debre Zeit				
Bed type \ var	ILL-590	Alemaya	Denbi	Derash	Mean
	PDI_f[1]	PDI_f [1]	PDI_f [1]	PDI_f [1]	
Flat bed	82.20^{g}	40.06^{de}	45.90^{e}	34.94^{a-d}	50.7
Raised bed	47.25^{e}	26.60^{ab}	28.67^{ab}	25.14^{a}	34.9
Farmers' practice	60.17^{f}	30.62^{a-d}	38.83^{c-e}	35.02^{a-d}	41.2
Tied ridge bed	72.61^{g}	28.03^{ab}	35.42^{b-d}	28.87^{a-c}	41.3
Mean	65.51	31.33	37.20	30.99	42.0
LSD (0.05)	4.9				
CV (%)	14.2				

[1]PDI_f = final percent of disease incidence of lentil Fusarium wilt at every 15 days interval; LSD =Least significant difference at $P \leq 0.05$; CV= Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

Table 8. Interaction effects of seedbed types and lentil varieties on Fusarium wilt (*F. oxysporium* f.sp. *lentis*) of final disease incidence (%) at Chefe Donsa, Ethiopia, during the 2017 main cropping season

Treatment combinations		Percent of disease incidence at every 15 days interval at Chefe Donsa			
Bedtype	varieties	ILL-590	Alemaya	Denbi	Derash
		PDI_f[1]	PDI_f[1]	PDI_f [1]	PDI_f [1]
Flat bed		67.5 ^{g}	35.1^{de}	39.8^{e}	31.2^{d}
Raised bed		52.5 ^{f}	11.4^{a}	20.5^{bc}	8.8 ^{a}
Farmers' practice		51.1 ^{f}	31.6^{de}	36.3^{de}	29.0^{cd}
Tied ridge bed		59.8^{fg}	16.8^{ab}	30.1^{cd}	15.6^{ab}
Mean		63.37	24.40	28.0	20.0
LSD (0.05)		11.2			
CV (%)		15.2			

[1] PDIf = final percent of disease incidence at every 15 days interval of lentil Fusarium wilt; LSD =Least significant difference at $P \leq 0.05$; CV = Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

The lowest values of 25.14 and 8.8% final Fusarium wilt incidence were obtained from planting the variety Derash on the raised seedbed type at Debre Zeit and Chefe Donsa, respectively (Tables 7 and 8). This indicated that integration of Derash variety with raised seedbed type lower wilt incidence than susceptible check with planting on the flat seedbed. The reason for such variation might be the removal of excess water from the raised beds that might have helped the drained plots to produce higher yield than the flat seedbed and enhanced the movement of required soil moisture through the root system. This result agrees with the observations made by other researchers (Srivastava *et al.,* 2000) who indicated that many attempts were made to manage this disease using cultural, varietal, biological and chemical methods.

The present findings also revealed that the epidemic of lentil Fusarium wilt was slightly varied between D/Z and C/D in 2017. This might be due to variation in environmental factors, pathogen population across the plots and associated amount and rainfall time and soil temperature (Appendix Table 6). Andrabi *et al*. (2011) reported that environmental factors, such as moisture and temperature, influenced the development of pathogens. Similar results were reported by Navas-Cortes *et al*. (2001) on Fusarium wilt incidence that was also positively correlated with increasing soil temperature and might be due to the inoculum density of the pathogen. In similar

work, Mehmood *et al*. (2013) found that rainfall and soil variables, especially temperature and moisture, had a significant positive effect on Fusarium wilt. Muhammad (2010) also reported a direct correlation between inoculum density and wilt severity in chickpea. According to Landa *et al*. (2006), temperature has a significant influence on the development of disease, metabolic process and plant development and pathogen. And also, poor soil aeration adversely affects root growth and root growth declines as soil oxygen depletes because of reduced cell division and expansion, which agrees with the suggestion of Braunack and Dexter (1989).

4.5.3. Area Under Disease Progress Curve (AUDPC)

Analysis of variance showed significant ($P \leq 0.05$) difference for the main effect and interaction effect of seedbed types and lentil varieties on area under disease progress curve (%-days) at both locations (Appendix Table 2).

Table 9. Interaction effects of seedbed type and lentil variety on Fusarium wilt (*F.oxysporium* f.sp. *lentis*) area under disease progress curve (%-days) at Debre Zeit, Ethiopia, during the 2017 main cropping season

Treatment combinations		Area under Disease Progress Curve (%-days) at Debre Zeit				
Bed type:	Var.	ILL-590	Alemaya	Denbi	Derash	Mean
Flat bed		1018^{h}	535.3^{e}	620.4^{f}	477.3^{de}	662.7
Raised bed		533.7^{e}	360.0^{a}	387^{ab}	342.4^{a}	405.8
Farmers' practice		789.8^{g}	402.2^{a-d}	512.3^{e}	455^{b-e}	539.8
Tied ridge bed		786.5^{g}	388.5^{a-c}	469^{c-e}	389^{a-c}	508.1
Mean		782.0	421.5	497.2	415.7	529.1
LSD (0.05)				81.2		
CV (%)				10.2		

LSD = Least significant difference at $P \leq 0.05$; CV= Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

The highest (1018.0; 899.4 %-days) AUDPC values were obtained from the integration of flat seedbed and ILL-590, while the lowest (342.4; 114.4 %-days) in AUDPC values were obtained from the integration of raised seedbed with the variety Derash at Debre Zeit and Chefe Donsa, respectively (Tables 9 and 10). The overall results indicated that integrated resistance and /moderately resistace variety and raised seedbed type practice was effective to slow down the epidemics of fusarium wilt and to sustain lentil production and productivity which confirmed with the finding of Negussie *et al*., (2006) and Palti and Katan, (1997) reported that substantial

reductions in plant mortality due to wilt/root rots were recorded when a combination of moderately resistant varieties and drainage methods that was raised seedbed type was used.

Table 10. Interaction effects of seedbed type and lentil variety on Fusarium wilt (*F.oxysporium* f.sp. *lentis*) of area under disease progress curve (%-days) at Chefe Donsa, Ethiopia

Treatment combinations		Area under Disease Progress Curve (%-days) at Chefe Donsa				
Bed type:	Var.	ILL-590	Alemaya	Denbi	Derash	mean
Flat bed		899.4^{h}	396.0^{cd}	518.0 ^{e}	415.2^{d}	561
Raised bed		679.0^{fg}	155.5^{a}	295.0^{bc}	114.0^{a}	326
Farmers' practice		679.2^{f}	423.0^{de}	446.0^{de}	352.0^{cd}	374
Tied ridge bed		752.6^{g}	207.0^{ab}	397.0^{cd}	215.0^{ab}	376
Mean		738.6	278.9	379.9	238.9	
LSD (0.05)				62.9		
CV (%)				11.0		

LSD = Least significant difference at P ≤ 0.05; CV= Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

Similarly, Merkuz and Getachew (2012a) reported that growing resistant and moderately resistant varieties on raised seedbed that drain excess water with recommended seeding rate could reduce plant mortality caused by chickpea wilt. There was Fusaruim wilt pressure on the susceptible line and high inoculum pressure had major influence on disease development and reproduction in conformity with the reports of Est´evez *et al*., 2002; Abawi and Ludwig, 2005) reported that root diseases are most severe in susceptible crop varieties, because the pathogen inoculum can build up quickly when environmental conditions are conducive for disease development. Hence, the present study indicated that Fusarium wilt incidence might be minimized by careful selection of resistant lentil genotypes and raised seedbed type that serve as the most important agronomic factors to increase lentil productivity.

4.5.4. Disease Progress Rate and Curve

The disease progress rate was significantly different among varieties and seedbed practiced (Tables 11 and 12). The disease progress rates when planted on flat seedbed, raised seedbed, tie-ridge and farmers' practice were 0.0308, 0.0126, 0.0234 and 0.0146 units per day for the variety ILL-590; 0.00792, 0.00475, 0.00516 and 0.00689 units per day for Derash; 0.00803, 0.00456, 0.00874 and 0.00824 units per day for Alemaya; 0.00962, 0.00515, 0.00659 and 0.00649 units per day for Denbi, respectively, at Debre Zeit (Table 12), whereas the corresponding rates were

0.0249, 0.0107, 0.0103, and 0.0.0134 units per day for the variety ILL-590; 0.0.00699, 0.0.00400, 0.00565, and 0.00483 units per day for Derash; 0.00776, 0.0044, 0.00469 and 0.00512 units per day for Alemaya; 0.00821, 0.00432, 0.00499 and 0.0061 for Denbi respectively, at Chefe Donsa (Table 12).

Table 11. Disease progress rates due on lentil varieties and due to seedbed types at Debre Zeit in 2017 main cropping season

Treatments		Intercept	SE of Intercept	Progress Rate	R^2
Seedbed type	Variety				
Planting on the open raised bed type	ILL-590	- 0.169	0.179283	0.01260	55.6%
	Derash	- 0.0493	0.0256341	0.00475	93.0%
	Denbi	-0.0561	0.0268948	0.00515	88.6%
	Alemaya	- 0.0066	0.0239010	0.00456	92.7%
Planting on the farmer practice seedbed type	Denbi	- 0.0148	0.0589394	0.00649	79.3%
	ILL-590	- 0.137	0.0672447	0.0146	94.3%
	Derash	- 0.0030	0.0307974	0.00689	94.6%
	Alemaya	- 0.0006	0.0273233	0.00824	92.5%
Planting on the flat seedbed type	Denbi	- 0.0507	0.0849538	0.00962	81.8%
	Alemaya	- 0.0481	0.0361969	0.00512	94.5%
	ILL-590	- 0.629	0.233000	0.0308	96.0%
	Derash	- 0.0142	0.0240954	0.00792	96.1%
Planting on the tie- ridge bed type	Derash	- 0.0166	0.0245163	0.00516	94.0%
	Alemaya	-0.0066	0.0193596	0.00874	95.4%
	Denbi	- 0.0281	0.0292224	0.00659	94.7%
	ILL-590	- 0.566	0.247884	0.0234	75.9%

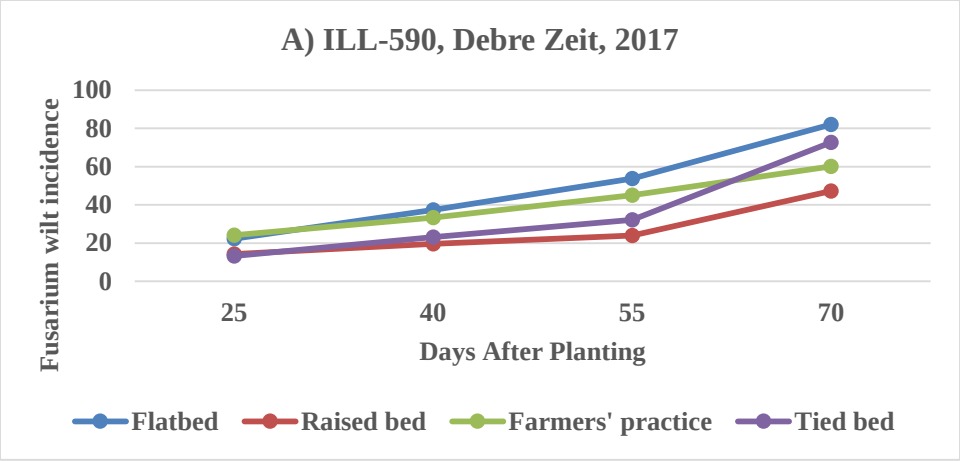
The disease progress rates showed variations among the seedbed types of cultural practices used in four lentil varieties and in the two testing locations. Disease progress rates calculated on the variety Derash for raised seedbed, tie-ridge seedbed, farmer practice and flat seedbed types ranged from 0.00475 to 0.00792 and 0.00400 to 0.00699 units per day at Debre Zeit and Chefe Donsa, respectively, from 0.00456 to 0.00874 and from 0.00475 to 0.00874 units per day for Alemaya variety at Debre Zeit and Chefe Donsa, respectively, whereas on the variety ILL-590, the rates ranged from 0.0126 to 0.0308 and from 0.0107 to 0.0249 units per day at Debre Zeit and Chefe Donsa, respectively and 0.0264 and 0.0375 units per day on the variety Denbi in 2017 cropping season (Tables 11 and 12). The disease progress rate was faster (0.0308) on the susceptible check, ILL-590 than on other varieties in all seedbed types.

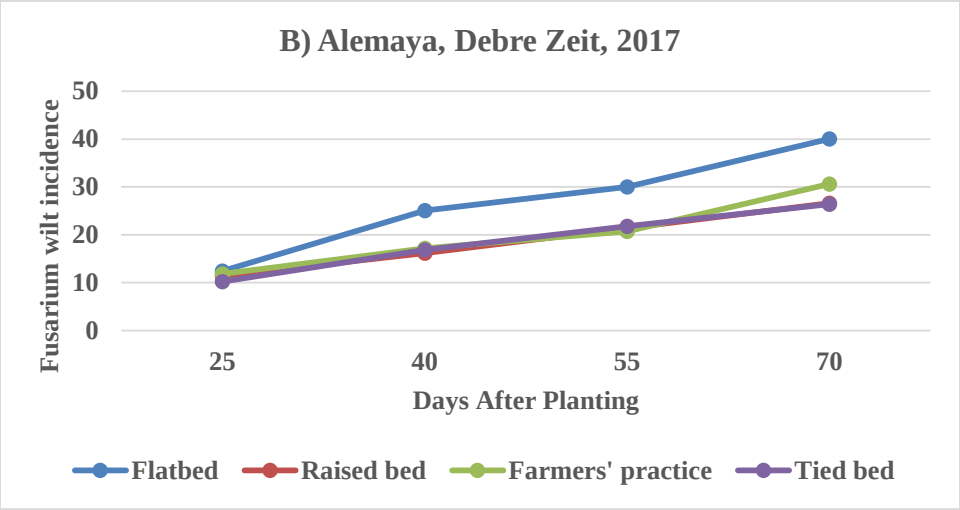
Table 12. Disease progress rate due to seedbed types and lentil varieties at Chefe Donsa in 2017 main cropping season

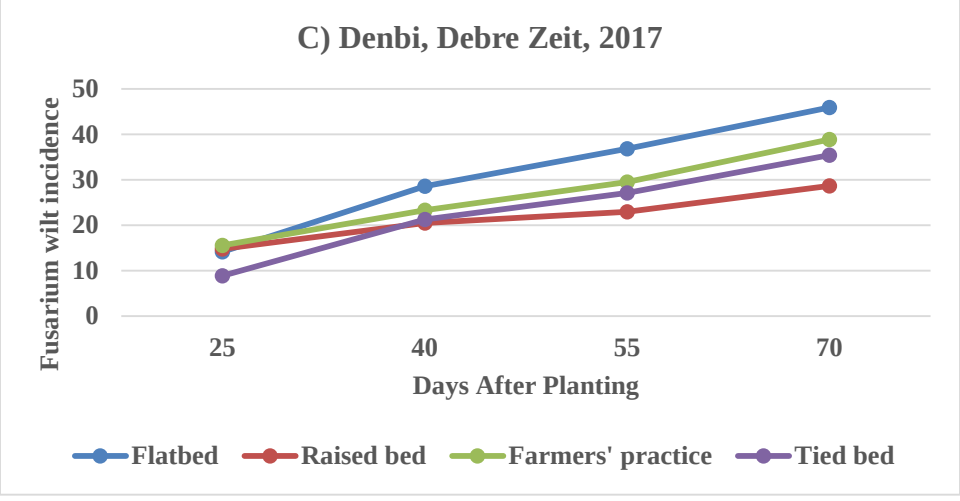
Treatment Seed bed types	Variety	Intercept	SE of intercept	Disease Progress Rate	R^2
Planting on the open raised bed type	ILL-590	- 0.254	0.15569	0.0107	89.9%
	Derash	- 0.0515	0.0387780	0.00400	71.3%
	Denbi	- 0.0420	0.0966788	0.00432	96.1%
	Alemaya	- 0.0367	0.086691	0.00440	72.1%
Planting on the farmer practice seedbed type	Denbi	- 0.0755	0.0715654	0.00616	94.3%
	ILL-590	- 0.064	0.190048	0.01340	63.0%
	Derash	- 0.107	0.15111	0.00483	79.1%
	Alemaya	- 0.137	0.18332	0.00512	71.5%
Planting on the flat seedbed type	Denbi	- 0.0526	0.141774	0.00821	64.6%
	Alemaya	- 0.159	0.14171	0.00776	81.3%
	ILL-590	- 0.253	0.31489	0.0249	84.2%
	Derash	-0.035	0.089524	0.00699	80.6%
Planting on the tie- ridge bed type	Derash	- 0.0101	0.111725	0.00565	77.3%
	Alemaya	- 0.152	0.322408	0.00469	65.4%
	Denbi	- 0.113	0.274120	0.00499	63.3%
	ILL-590	- 0.114	0.0931541	0.0103	85.6%

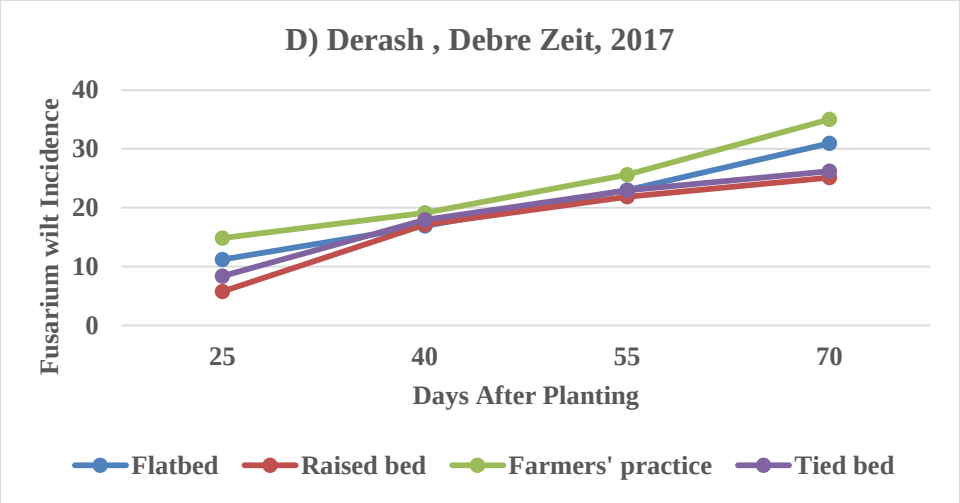
At Chefe Donsa, the rates varied from 0.00440 to 0.00776 units per day on Alemaya variety and from 0.00400 to 0.00699 units per day on the Derash variety, while rates ranged from 0.00456 to 0.00874 units per day for Alemaya variety and from 0.00475 to 0.00792 units per day on the variety Derash at Debre Zeit (Tables 11 and 12). Derash variety reduced rate of disease progress with the integration of raised bed type. Generally, the result showed that the wilt progress rate was slower on the lentil variety Derash, followed by Alemaya than on ILL-590 at both locations.

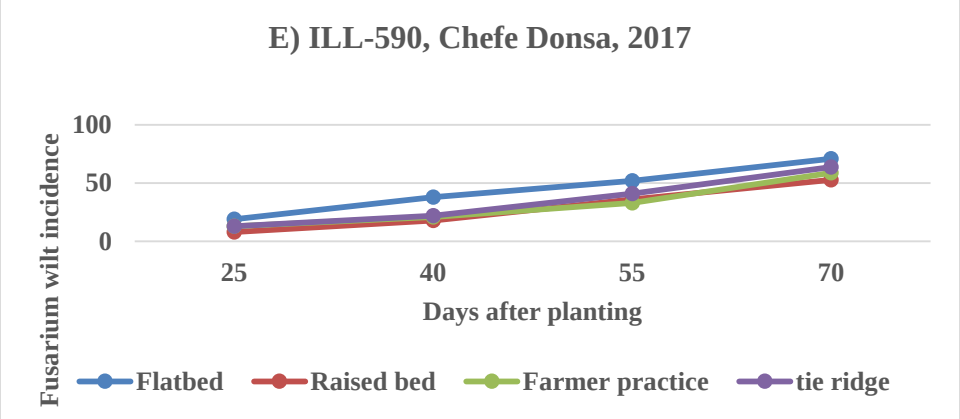
The disease progress curves of Fusarium wilt (incidence versus DAP) were sketched separately for each lentil variety (Figure 2). Each curve for all lentil varieties revealed that Fusarium wilt incidence progressed increasingly starting from the first wilt appeared to the final incidence recorded at both locations during the assessment periods. The disease progress curves on the four lentil varieties for four seedbed types were illustrated and were not similar for each seedbed type used. Fusarium wilt incidence level increased from the initial to final assessment date and the curves showed increasing trends in disease progresses for the four lentil varieties in each management practice (seedbed types) in all its assessments. However, the increasing trend in the raised seedbed method was comparatively lower than in the other seedbed types (Figure 2).

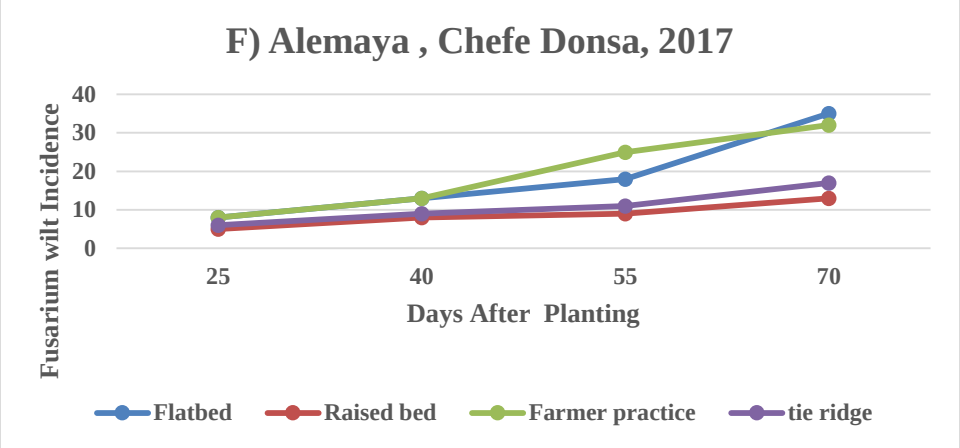

A) ILL-590, Debre Zeit, 2017
Fusarium wilt incidence
100
80
60
40
20
0
25
40
55
70
Days After Planting
Flatbed
Raised bed
Farmers' practice
Tied bed

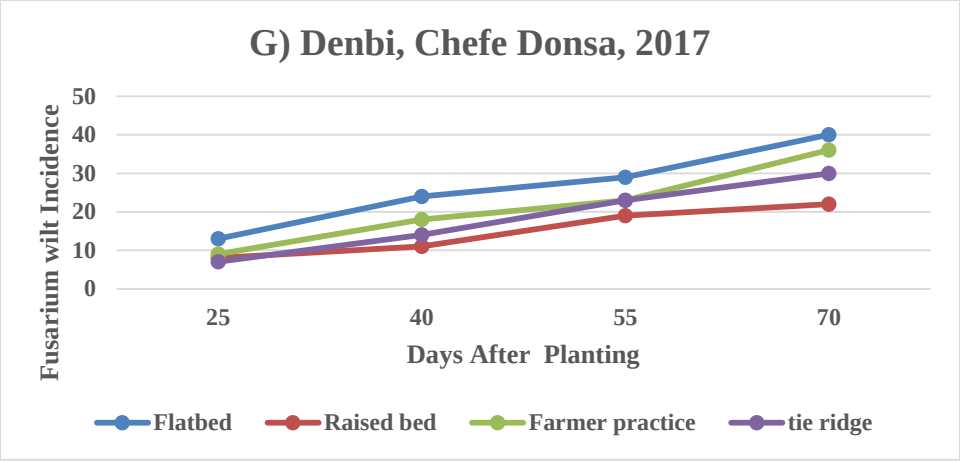

B) Alemaya, Debre Zeit, 2017
Fusarium wilt incidence
50
40
30
20
10
0
25
40
55
70
Days After Planting
Flatbed
Raised bed
Farmers' practice
Tied bed


C) Denbi, Debre Zeit, 2017
Fusarium wilt incidence
50
40
30
20
10
0
25
40
55
70
Days After Planting
Flatbed
Raised bed
Farmers' practice
Tied bed


D) Derash , Debre Zeit, 2017
Fusarium wilt Incidence
40
30
20
10
0
25
40
55
70
Days After Planting
Flatbed
Raised bed
Farmers' practice
Tied bed


E) ILL-590, Chefe Donsa, 2017
Fusarium wilt incidence
100
50
0
25
40
55
70
Days after planting
Flatbed
Raised bed
Farmer practice
tie ridge


F) Alemaya , Chefe Donsa, 2017
Fusarium wilt Incidence
40
30
20
10
0
25
40
55
70
Days After Planting
Flatbed
Raised bed
Farmer practice
tie ridge



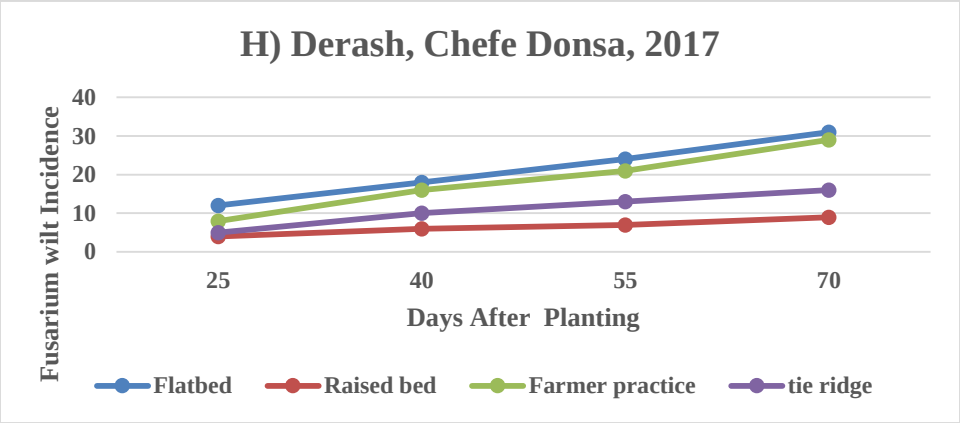


Figure 2. Fusarium wilt progress curves as influenced by seedbed types and lentil varieties at Debre Zeit and Chefe Donsa.

There was an increased Fusarium wilt incidence progress rate over time at both locations. The disease progress rate curve had more or less similar trend increased in wilt incidence but not uniform for all treatment combinations. This indicated that the maximum wilt progress rate was obtained from flat seedbed type integrated with the susceptible genotype while minimum was computed from the intehration of cv. Derash with the raised seedbed type throughout the disease assessment frequency starting from the onset of initial wilt incidence. Disease incidence on the plots of the susceptible genotype, ILL-590 had high progressive curves and displayed the highest level of Fusarium wilt incidence. However, the lowest Fusarium wilt incidence was calculated for the moderately resistance cv. Derash when planted on raised seedbed cultural practice.

On the other hand, the highest Fusarium wilt incidence was observed throughout the disease recording fifteen days interval starting from the first wilt symptom appeared 25 days after

planting (DAP). The present findings showed both the varietal difference and seedbed types made the variation in each seedbed type and four varieties used. Ingeneral, the the disease progress was lower in the integration of cv. Derash with the raised bed type than the others.

4.5.5. Effect of Seedbed Types and Varieties on Days to 50% Flowering and Days to 90% Physiological Maturity

The analysis of variance (ANOVA) showed that only the main effect of varieties had highly significant ($P \leq 0.01$) difference on days to 50% flowering at both locations (Appendix Table 3 and 4). The main effect of seedbed types was significant ($P \leq 0.05$) at Chefe Donsa, while non-significant ($P \leq 0.05$) difference at Debre Zeit. And also there were no significant ($P \leq 0.05$) difference along the interactions among lentil varieties and seedbed types on days to 50% flowering at both sites (Appendix Tables 3 and 4). Similarly, Edossa *et al*. (2010) reported that no significant variation was recorded for some traits, like seed weight per plant and days to flowering. This might be attributed to the fact that days to flowering in lentil were considered to be varietal characteristics, which is genetically controlled. Previous studies showed that, the differential response to flowering among varieties was distinct. According to Matrne and Siddique, (2009) flowering time is determines length of vegetative phases or sowing to flowering and also climatic conditions that the crop will be exposed during reproductive growth.

Only the main effect of varieties for the days to 90% physiological maturity was significantly ($P \leq 0.05$) different at Debre Zeit; however, the main effects of seedbed types and lentil varieties as well as their interactions were non significant ($P \leq 0.05$) different for days to 90% physiological maturity at both locations (Appendix Tables 3 and 4).

4.5.6. Effect of Seedbed Types and Varieties on Lentil Aboveground Biomass

Aboveground biomass had highly significant ($P \leq 0.01$) difference in the main effects and interaction effects of seedbed types and lentil varieties at both sites (Appendix Tables 3 and 4).

Higher aboveground biomass (1233.3 : 1767 g per plot) was obtained by the integration of raised seedbed with Derash variety at Debre Zeit and Chefe Donsa, respectively, and reduced Fusarium wilt incidence more than the other treatment combinations (Tables 13 and 14). Relative to the flat seedbed, aboveground biomass yield was increased by 87.52% and 40.16% in the raised bed

type used at Debre Zeit and Chefe Donsa, respectively. Similarly, aboveground biomass increased more in Derash variety than the susceptible lentil line.

The lowest 196.67 and 326.67 g per plot aboveground biomass weights of lentil were obtained from the plots planted with ILL-590 genotype on the flat seedbed type, whereas the highest 1233.3 and 1766.67 g per plot lentil biomass weights were harvested from plots planted with the variety Derash in their integration with raised seedbed type at Chefe Donsa and Debre Zeit, respectively, (Tables 13 and 14).

Table 13. Interaction effects of seedbed types and lentil varieties on aboveground biomass at Debre Zeit in 2017 main cropping season

Treatment combinations	Aboveground biomass weight of lentil (gram per plot) at Debre Zeit				
	ILL-590	Alemaya	Denbi	Derash	Mean
Flat bed	196.7^{a}	550.0^{ab}	635.0^{bc}	598.3^{bc}	527.5
Raised bed	523.3^{ab}	810.0^{c}	1232.3^{d}	1233.3^{d}	950.0
Farmers' practice	328.3^{a}	710.0^{bc}	666.7^{bc}	676.7^{bc}	595.4
Tie ridge bed	506.7^{ab}	1050.0^{d}	1216.7^{d}	1183.3^{d}	989.2
Mean	421.3	780.0	937.9	922.9	
LSD (0.05)	228.2				
CV (%)	15.5				

LSD =Least significant difference at $P \leq 0.05$; CV-Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

Table 14. Interaction effects of seedbed types and lentil varieties on aboveground biomass at Chefe Donsa in 2017 main cropping season

Treatment combinations	Aboveground biomass weight of lentil (gram per plot) at Chefe Donsa				
	ILL-590	Alemaya	Denbi	Derash	mean
Flat bed	326.67^{c}	1000.0^{a}	1053.3^{a}	1167.0^{a}	854.2
Raised bed	623.0^{a}	1367.0^{ab}	1433.0^{ab}	1767.0^{b}	1197.4
Farmers' practice	423.33^{a}	1200.0^{a}	1216.7^{a}	1500.0^{ab}	1085.0
Tie ridge bed	760.0^{a}	1200.0^{a}	1100.0^{a}	1467.0^{ab}	1006.7
Mean	275.75	1191.67	1200.83	1475.0	
LSD (0.05)	228.2				
CV (%)	15.5				

LSD =Least significant difference at $P \leq 0.05$; CV-coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

4.5.7. Effect of Seedbed Types and Lentil Varieties on the Mean Grain Yield

Highly significant ($P \leq 0.01$) differences were obtained in the main effects and the interaction effects of seed bed type practiced and lentil varieties at Debre Zeit and Chefe Donsa (Appendix Tables 3 and 4).

Table 15. Interaction effects of lentil varieties and seedbed types on mean grain yield at Debre Zeit, Ethiopia, during the 2017 main cropping season

Treatment combinations	Mean grain yield of lentil (kg ha^{-1}) at Debre Zeit				
Var.	ILL-590	Alemaya	Denbi	Derash	Mean
Flat bed	64.4^{a}	935.0^{cd}	733.7^{abc}	807.3^{abc}	185.1
Raised bed	193.8^{abc}	1898.4^{e}	1740.6^{e}	1882.5^{e}	735.5
Farmers' practice	179.2^{abc}	1150.6^{d}	927.1^{bcd}	986.7^{cd}	360.89
Tie ridge bed	80.2^{ab}	1509.0^{e}	1167.5^{d}	1662.7^{e}	598.23
Mean	129.38	669.27	467.24	613.78	
LSD (0.05)	273.4				
CV (%)	15.5				

LSD =Least significant difference at $P \leq 0.05$; CV-Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

Table 16. Interaction effects of lentil varieties and seedbed types on mean grain yield at Chefe Donsa, Ethiopia, in the 2017 main cropping season

Treatment combinations	Mean grain yield of lentil (kg ha^{-1}) at Chefe Donsa				
Var.	ILL590	Alemaya	Denbi	Derash	Mean
Flat bed	68.0^{a}	2151.0^{b}	2448.0^{b-d}	2593.0^{c-e}	1815.03
Raised bed	164.0^{a}	2893.0^{de}	3268.0^{g}	3827.0^{h}	2489.79
Farmers' practice	119.0^{a}	2700.0^{ef}	2298.0^{bc}	3130.0^{fg}	2109.99
Tie ridge bed	90.0^{a}	2581.0^{c-e}	2626.0^{c-e}	3163.0^{fg}	2114.92
Mean	110.3	2581.3	2659.9	3178.3	2132.43
LSD (0.05)	331.7				
CV (%)	8.9				

LSD =Least significant difference at $P \leq 0.05$; CV-Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

The interaction effect indicated the highest, 3827.0 and 1882.5 kg ha^{-1} mean lentil grain yields obtained from the variety Derash planted on raised seedbed type at Chefe Donsa and Debre Zeit, respectively (Tables 15 and 16). And also (2893.0 and 1898.4 kg ha^{-1}) were obtained from plots

where Alemaya variety was planted on raised seedbed type at Chefe Donsa and Debre Zeit, respectively (Tables 15 and 16). But, the lowest 64.4 and 68.0 kg ha^{-1} mean grain yield of lentil were obtained from the integration of ILL-590, susceptible line with flat seedbed type at Debre Zeit and Chefe Donsa, respectively (Tables 15 and 16).

Derash variety produced high (3827.0 kg ha^{-1}) mean grain yield on raised seedbed type and generally showed better performance on all seedbed types than other varieties tested. Similarly, growing lentil crops on raised seedbed produced significantly superior agronomic characteristics; yield attribute traits, seed and straw yields as compared to the flat bed sown crop (Rathore *et al.*, 2010). Absolutely this interpretation indicated that the best management option to reduce the wilt problem was approaches to the use of improved varieties with resistance to wilt. Potential mean grain yields (3827.0, 3268.0 and 2893.0 kg $ha^{-1)}$ of lentil were obtained when the varieties Derash, Denbi and Alemaya were integrated with raised seedbed type at Chefe Donsa, respectively (Table 16). Similarly, the increased mean grain yield was obtained from the plots of Alemaya, Derash and Denbi varieties integrated with raised seedbed type whose mean seed yields were (1898.4, 1882.5, and 1740 kg $ha^{-1)}$, significantly out yielded the susceptible check, ILL-590 (64.4 kg ha^{-1}) at Debre Zeit, respectively (Table 15).

Generally, maximum (3827.0 kg ha^{-1}) lentil yield with least wilt incidence (8.8%) was obatianed in the plots sown with the variety Derash integrated with raised seedbed type compared to flat bed at Chefe Donsa (Table 16). And also cv. Derash was advanced by 133.18% of mean grain yield with the integration of raised bed type compared to the flatbed at Debre Zeit. This result is in agreement with the results of Schulthess *et al.* (1997) who reported a significant increase in lentil grain yield Vertisols with the appropriate seedbed type and improved variety used. Similarly, Abate *et al*. (1993) reported 58% yield increase in durum wheat and 106% in chickpea and lentil were obtained when planted on raised bed over planting on flatbed.

4.5.8. Correlations of Disease Incidence and AUDPC with Growth and Yield

Correlation coefficient between yield and yield parameters was strong, positive and highly significant ($P \leq 0.01$) at both locations (Tables 17 and 18).

Correlation coefficients (r) found significant differences on disease incidence and AUDPC with yield and yield components. The association of disease incidence was negatively correlated with 50% of days to flowering, seed grain yield and biomass yield. Highly significant ($P \leq 0.01$) and strong positive correlation existed between aboveground biomass and grain yield. These results were in agreement with findings of Anjam *et al*. (2005) who reported that the increase in biomass would have a positive and significant effect onsgrain yield. Similarly, Singh *et al*. (1989) reported that plant height, plant biomass, branches/plant, and days to maturity resulted in significant positive correlation with grain yield.

There was highly significant ($P \leq 0.01$) and negatively correlated between disease incidence with seed grain yield and biomass yield of lentil at both locations. The same was true for area under disease progress curve that exhibited highly significant ($P \leq 0.01$) and negatively correlated with seed grain yield and biomass yield. Positive association was calculated between days to 90% physiological maturity and seed yield. Aboveground biomass and grain yield were negatively correlated to disease parameters at both locations (Tables 17 and 18).

Yield components (days to 50% flowering, days to 90% physiological maturity, aboveground biomass, grain yield) showed a negative correlation with wilt incidence at the assessment times (Tables 17 and 18). This indicated that the higher wilt incidence result the lower lentil aboveground biomass and seed grain yield. Similarly, there was a strong negative correlation between Fusarium wilt incidence and seed grain yield, which was estimated at 8.8% yield loss for every 10% Fusarium wilt incidence (Erskine and Bayaa, 1996). The amount of Fusarium wilt incidence recorded in the two locations varied considerably, possibly because of differences lentil genotypes performance, environmental conditions and pathogen population in the soil across location as a result of increase in disease in successive lentil sowings.

Days to 50% flowering, biomass had a significant and negative correlation with Fusarium wilt incidence and area under disease progress curve at both locations (Tables 17 and 18). A day to 90% of maturity was significant and negatively correlated with disease parameters at Debre Zeit. However, was non- significant and negatively correlated with disease parameters at Chefe Donsa. This result is in agreement with the results of Whitehead *et al*. (2000) who reported a strong and positive correlation was obtained between lentil seed yield and biomass.

Table 17. Correlation coefficient (r) between disease incidence and AUDPC values with growth and yield components of lentil at Chefe Donsa during 2017 main cropping season

Trait	PCE	DAP25	DAP40	DAP55	DAP70	AUDPC	DF	DM	BM	YLD
PCE										
DAP25	-0.138^{ns}									
DAP40	-0.163^{ns}	0.916**								
DAP55	-0.099^{ns}	0.850**	0.908**							
DAP70	-0.018**	0.812**	0.849**	0.939**						
AUDPC	-0.029^{ns}	0.840**	0.886**	0.978**	0.990**					
DF	0.0959^{ns}	-0.623**	-0.662**	-0.781**	-0.846**	-0.832**				
DM	0.026^{ns}	-0.250^{ns}	-0.208^{ns}	-0.118^{ns}	-0.102^{ns}	-0.110^{ns}	0.058^{ns}			
BM	0.147^{ns}	-0.660**	-0.700**	-0.840**	-0.894**	-0.885**	0.840**	0.04^{ns}		
YLD	0.361^{ns}	-0.666**	-0.694**	-0.824**	-0.906**	-0.886**	0.895**	0.02^{ns}	0.96**	

PCE=Plant count at emergence; DAP=Days after planting: DF= 50% of flowering: AUDPC= Area under Disease Progress Curve: DM= 90% of physiological maturity: BM= biomass: YLD= Yield; * = significant, ** = highly significant and ns = non significant.

Table 18. Correlation coefficient (r) between Fusarium wilt incidence and AUDPC values with yield and yield components of lentil at Debre Zeit during 2017 main cropping season

Trait	PCE	DAP25	DAP40	DAP55	DAP70	AUDPC	DF	DM	BM	YLD
PCE										
DAP25	-0.092^{ns}									
DAP40	-0.001**	0.8291**								
DAP55	-0.010**	0.7682**	0.9055**							
DAP70	- 0.057*	0.7082**	0.7879**	0.7312**						
AUDPC	-0.014*	0.8070**	0.9293**	0.9410**	0.8016**					
DF	0.180^{ns}	-0.374*	-0.381*	-0.452*	-0.632**	-0.48**				
DM	0.174^{ns}	-0.339*	-0.337*	-0.381**	-0.619**	-0.42**	0.91**			
BM	0.014*	-0.686**	-0.599**	-0.573**	-0.699**	-0.663**	0.567**	0.53**		
YLD	0.110^{ns}	-0.694**	-0.67**	-0.624**	-0.752**	-0.718**	0.41**	0.31*	0.80**	

PCE=Plant count at emergence; DAP=Days after planting: DF= 50% of flowering: AUDPC= Area under Disease Progress Curve: DM= 90% of physiological maturity: BM= biomass: YLD= Yield. **, Correlation is significant at the 0.01 level (significantly different at 1% probability level and * Correlation is significantly different at 5% probability level; * = significant, ** = highly significant and ns, non-significant.

5. SUMMARY AND CONCLUSIONS

Lentil is one of the most nutritious pulse crop next to chickpea, and a valuable human food for resource poor farmers. It also a major cash crop and farmers earn high income. In Ethiopia, lentil is produced under a wide range of altitude from 1600 to 2700 m.a.s.l. mainly in main season. Due to several biotic and abiotic factors, lentil production and productivity in Ethiopia has been low. Of all constraints, lentil wilt caused by *Fusarium oxysporum,* was one of the most economically important that tackle the lentil farming systems in studied districts. The objectives of this study were to assess the distribution of Fusarium wilt of lentil in central highlands of Ethiopia; to evaluate the effect of cultivars and seedbed types as a component of integrated management option and to determine the extent of seed infection due to Fusarium wilt of lentil on seed lots collected from different sources in central highlands of Ethiopia.

To find out the prevalence, distribution and incidence of the disease on farmer's field a planned survey of the lentil growing area was carried out. Atotal of 192 fields were inspected from 8 districts *viz.*, Ada'a, Aleltu, Ensaro, Gimbichu, Lume, Moretina-Jiru, Minjar and Siyadebrena-Wayu. In all the locations surveyed none of the field remained free from the wilt disease. The incidence of wilt in the surveyed fields ranged from 2 to 75% in villages. The minimum average incidence of wilt was observed in Siyadebrena-wayu (13.2%) followed by Ada'a (14.5%). Maximum wilt incidence was observed in Moretina-Jiru (32.8%) followed by Aleltu (28.0%).

The survey showed that incidence of lentil wilt varied from village to village due to factors like diverse management practice, temperature, soil moisture, rainfall, sowing dates, diverse cultivars used and even it could also be attributed to existence of pathogenic variability. The higher disease incidence may be due to susceptibility of the cultivars or favorable environmental conditions and lack of appropriate seedbed used in the excess soil moisture. Higher wilt incidence was computed where the crop was planted in the poor drainage soil moisture than others likely reflecting the wet conditions that favor disease development. Symptoms were present at all physiological stages with varying degrees from one field to another and from one area to another. Identification of cultural and morphological studies showed that apparently maximum (100%) frequency of *Fusarium oxysporum* fungi was associated with all collected infected lentil plant samples followed by *Rhizoctonia* spp. (24.4%). *Sclerotium rolfsii* (3.0%).

Evaluation of seed infection was conducted in Plant Pathology Laboratories at DZARC. A total of 100 seed samples were collected, 30 seed samples from each farmers' saved, and open markets and 40 seed samples from Agricultural Research Centers. PDA media was used to detect level of seed infections. The highest (22.2%) level of seed infection was recorded on seed sample code 048 collected from open markets followed by seed sample code 057 (20.0%) and nil (0%) seed infection level by Fusarium wilt also observed on the most of collected samples.

The interactions effects of varieties by seedbed types showed significant ($P \leq 0.05$) difference. The highest (82.2% and 67.5%) final Fusarium wilt incidence was obtained by planting susceptible check, ILL-590 on flat seedbed type while the lowest 25.14% and 8.8% final wilt incidence was obtained from the integration of variety Derash with raised seedbed at Debre Zeit and Chefe Donsa, respectively. The highest (782%-days) AUDPC was obtained on the susceptible genotype, ILL-590, followed by Denbi variety (705%-days). The AUDPC clearly indicated varietal difference between the treatments. The Fusarium wilt disease rate progressed rapidly on genotype ILL-590 with flatbed than on the others. Except at the first wilt symptom appeared, (25 DAP) the main effects of varieties and seedbed types showed a highly significant ($P \leq 0.01$) difference on Fusarium wilt incidence, AUDPC, biomass, mean seed grain yield among the treatments at both locations. Association of disease incidence with yield and yield components was negatively correlated and significant and inverse relationship.

The highest (3827.0 kg ha^{-1} and 1882.5 kg ha^{-1}) mean grain of lentil with the least (8.8%) and (25.14%) Fusarium wilt incidence was obtained from the integration of cv. Derash with the raised seedbed type Chefe Donsa and Debre Zeit, respectively, where as the lowest (64.4 kg ha^{-1} and 68.0 kg ha^{-1} mean grain of lentil was computed from the integration of ILL-590 genotype and flatbed at Debre Zeit and Chefe Donsa.

Generally, from the result of the experimental test carried out at Chefe Donsa site the uses of raised seedbed type integrated with improved variety (Derash) will no doubt to improve lentil production and reduce the lentil wilt disease. For Debre Zeit Alemaya with raised bed was suggested. Thus, planting improved lentil variety on the raised seedbed type to reduced Fusarium wilt should be regarded as one facet of the integrated control program rather than used alone. In this current study, it was observed that moderately resistance variety and raised seedbed type

reduced disease parameters of lentil Fusarium wilt. Possibly these treatment combination could enhance the health and vigority of plants that might increase plant chances to withstand pathogen attack and to activate the host defense system.

Thus, it concluded that extreme distribution and high level of lentil wilt incidence was observed and also detection of lentil seed confirms that seed from different source harboring low level of inoculums sources of the pathogen and mechanism for long distance dispersal across region through seed scheme. Particularly, those farmers whose used seed shared from opened market play great role for source of inoculum in the next cropping season. Not only that across research centers and farmers saved seed the pathogen can be transmitted. Due to the absence of strong seed certification and seed health testing in Ethiopia it leads to infested seed being dispatched to farmers. Hence, it was suggested that to overcome these constraints more efforts need to be taken though farmers field and scale up of the improved lentil varities to increase its adoption for the producers. Improved lentil cultivar (Derash) that are moderately resistance to Fusarium wilt integrated with the raised bed type should be provided as an integrated management option for the studied areas.

Even though these research results dealt with a one year data and only over two testing locations, it is important to reduce Fusarium wilt and distribution as well as to optimize the seed grain yield production and productivity through use of the variety Derash followed by Alemaya than other used varieties with raised seedbed type. The future studies will be strongly support the use of improved varieties and appropriate seedbed type (raised seedbed) to make well drainage of excess soil moisture and adoption of existing improved varieties per agro-ecology. Also, the research should be focus on the screening of germplasm for the source of wilt resistance and existence of pathogenic variability across the country should be further studied.

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7. APPENDICES

Appendix Table 1. Analysis of variance for the main effects and interaction effects of seedbed types and lentil varieties on percent of disease incidence (PDI) at Debre Zeit and Chefe Donsa during 2017 main cropping season

Source varitio	Mean square of disease incidence (%) at **Debre Zeit**					Mean square of disease incidence (%) at **Chefe Donsa**			
	DF	DAP^{25}	DAP^{40}	DAP^{55}	DAP^{70}	DAP^{25}	DAP^{40}	DAP^{55}	DAP^{70}
Rep	2	4.2^{ns}	10.7*	8.0^{ns}	28.0^{ns}	6.8^{ns}	6.9^{ns}	3.8^{ns}	1.1*
B	3	92.9**	236.9**	519.7**	708.3**	93.4**	340.5**	375.7**	894.9**
Rep*B	6	2.74^{ns}	2.8^{ns}	13.3^{ns}	42.2^{ns}	1.8^{ns}	14.7*	21.7*	63.8*
V	3	161.7**	271.0**	530.9**	3235**	105.0**	484.4**	1610**	3351**
B*V	9	10.3**	26.8**	68.1**	103.9*	8.5**	32.6**	78.5**	78.25**
Error	24	2.1	2.8	17.9	34.0	3.1	5.58	7.51	22.6
Total	47								

DAP^{25}: DAP^{40}: DAP^{55} and DAP^{70}= Assessment of disease incidence at first, second, third and fourth days after planting score respectively. B = Bed type: B*V= Interaction of bed and variety: V=Lentil variety * Significant ($P < 0.05$), ** highly significant ($P < 0.01$) and ns, non-significant.

Appendix Table 2. Analysis of variance for the main effects and interaction effects of seedbed types and lentil varieties on area under disease progress curve (%-days) at Debre Zeit and Chefe Donsa during 2017 main cropping season

Source of variation	**Debre Zeit**				**Chefe Donsa**		
	DF	**$AUDPC^1$**	**$AUDPC^2$**	**$AUDPC^3$**	**$AUDPC^1$**	**$AUDPC^2$**	**$AUDPC^3$**
Rep	2	1533.3*****	2055.7^{ns}	3444.5^{ns}	1427.9*	1176.0^{ns}	330.08^{ns}
B	3	34319.2******	81906.3******	134455.5******	44431.8**	80311.7**	136307**
Rep*B	6	165.5^{ns}	1171.1^{ns}	2367.0^{ns}	1074.5^{ns}	3200.8*	8189.3**
V	3	47853.3******	86922.4******	357644.6******	58501.9**	216543**	539062**
B*V	9	3260.1******	9803.1******	13636.7******	3556.2**	10220.8**	15312.1**
Error	24	277.7	1560.9	2369.9	754.1	1043.6	1983.7
Total	47						

DF = degree of freedom; B= bed type; V= Lentil variety; =AUDPC = area under disease progress curve of lentil Fusarium wilt, * significant ($P < 0.05$), ** highly significant ($P < 0.01$) and ns, non-significant.

Appendix Table 3. Analysis of variance of data for main effects and interaction effects of seedbed types and lentil varieties on plant count at emergence (PCE), 50% days flowering(DF), days to 90% physiological maturity (DM), aboveground of biomass (BM) and grain yield (GY) at Debre Zeit during 2017 main cropping season

Source of var.	DF	**PCE**	**DF**	**DM**	**BM**	**GY**
Rep	2	982.3^{ns}	11.89*	33.33^{ns}	28819.27^{ns}	401.81^{ns}
B	3	711.7^{ns}	3.52^{ns}	22.77^{ns}	678557.46 **	719898.29**
Rep*B	6	874.5^{ns}	2.56^{ns}	11.69^{ns}	14682.46^{ns}	21325.66^{ns}
V	3	891.6^{ns}	84.52**	893.05**	692903.29**	705654.32**
B*V	9	340.7^{ns}	2.02^{ns}	38.09^{ns}	49605.15*	76389.50**
Error	24	922.9	2.9	30.1	20081.3	23674.0
Total	47					

PCE= plant count at emergence, DF= days to 50% flowering; DM=days to 90% physiological maturity, BM=aboveground of biomass per plot in (g) and GY=grain yield (kg ha^{-1}); * significant ($P < 0.05$), ** highly significant ($P < 0.01$) and ns, non-significant.

Appendix Table 4. Analysis of variance of data for main effects and interaction effects of seedbed types and lentil varieties on plant count at emergence (PCE), 50% days to flowering(DF), days to 90% physiological maturity (DM), aboveground of biomass (BM) and grain yield (GY) at Chefe Donsa during 2017 main cropping season

Source	**DF**	**PCE**	**DF**	**DM**	**BM**	**GY**
Rep	2	8.0^{ns}	6.58**	9.19^{ns}	40620.8^{ns}	12518.98^{ns}
B	3	3261.5*****	4.29*	14.79^{ns}	249520.0**	917034.28**
Rep*B	6	888.7^{ns}	0.9^{ns}	8.38^{ns}	36512.48^{ns}	48383.17^{ns}
V	3	1450.7*	250.0**	19.52^{ns}	3288412.2**	22650957.87**
B*V	9	2528.7*****	1.92^{ns}	25.13^{ns}	38703.17**	232611.86**
Error	24	956.5	1.3	49.6	31047.9	35949.40
Total	47					

PCE= plant count at emergence, DF= days of 50% flowering; DM=days to 90% physiological maturity, BM=aboveground of biomass per plot in (g) and GY=grain yield (kg ha^{-1}); * significant ($P < 0.05$), ** highly significant ($P < 0.01$) and ns, non-significant.

Appendix Table 5. Analysis of variance of data for survey data reflecting the biophysical associations with wilt

Variables	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Pesticide	1	214.4814	214.4814	2.241037	0.136438
fertilzer	2	1079.761	539.8807	5.641015	0.004322
Crop.stage	3	1425.43	475.1433	4.964596	0.002569
Planting.Date	2	2915.786	1457.893	15.23299	9.19E-07
S.Rate.kg/ha	5	727.4602	145.492	1.520193	0.186606
weed.contr	1	136.8067	136.8067	1.429442	0.233693
Variety	2	737.0417	368.5209	3.850539	0.023345
Pr.Crop	9	1299.501	144.389	1.508668	0.14931
land.prep	1	531.9233	531.9233	5.55787	0.019655
Drainage	3	1076.782	358.9272	3.750298	0.012314
Sol.Type	2	10.51944	5.25972	0.054957	0.946545
ploughing	2	756.8399	378.4199	3.95397	0.021157
Residuals	154	14738.77	95.70632	NA	NA

DF= Degree of freedom; sq= square; NA=not applicaple; pr=previous

Appendix Table 6. Monthly mean temperature (ºC) and monthly total rainfall (mm) at Debre Zeit and Chefe Donsa, Ethiopia, during lentil growing periods in 2017 main cropping season.

Croppin	Mean of temperature (ºC)								Monthly rainfall (mm)			
g month	**Debre Zeit**				**Chefe Donsa**				**Debre Zeit**		**Chefe Donsa**	
	2016		2017		2016		2017		2016	2017	2016	2017
	Max	Min	Max	Min	Ma x	Min	Ma x	Mi n	Mean (mm)	Total (mm)	Mean (mm)	Total (mm)
August	27.2	11.1	21.8	14.3	21	12	21	14	205.6	200.2	231.5	252.70
September	26.3	11.1	24.5	14.0	23	17	21	15	101.2	115.2	119.3	165
October	27.0	10.9	26.5	11.1	24	16	23	15	13.2	0.0	44.8	89.5
November	27.4	12	26.1	8.3	26	15	24	15	7.6	0.0	9	32.3