CHICKPEA WILT (*FUSARIUM OXYSPORUM* F. SP. *CICERIS)* INTENSITY IN NORTH SHOA, ETHIOPIA, AND EVALUATION OF CHICKPEA GENOTYPES AGAINST THE PATHOGEN

MSc Thesis

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ADVISORS' APPROVEL SHEET

(Submission sheet -1)

This is to certify that the thesis entitled **"**Chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris)* intensity in North Shoa, Ethiopia, and evaluation of chickpea genotypes against the pathogen **"** in partial fulfillment for the degree of Master of Science with specialization in **Crop Protection** to graduate program of School of Plant and Horticultural Sciences, College of Agriculture, and is a record of original research carried out by **Kalkidan Wudu Mengistu**, under our Supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore I/We recommend that it be accepted as fulfilling the thesis requirements.

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We, the undersigned, members of the Board of examiners of the final open defense by **Kalkidan Wudu Mengistu** have read and evaluated her thesis entitled **"**Chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris)* intensity in North Shoa, Ethiopia, and evaluation of chickpea genotypes against the pathogen **"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree **Masters of Science** in plant sciences with Specialization in **Crop Protection**

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DEDICATION

I dedicate this thesis manuscript to Sister **Banchi Wudu** for nursing me with affection and love and for her dedicated partnership in the success of my life

DECLARATION

I declare that this thesis is my bonafide work and all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name: Kalkidan Wudu_____ Signature _____

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CFW	Chickpea Fusarium Wilt
CSA	Central Statistical Agency of Ethiopia
DAI	Days after inoculation
DZARC	Debre Zeit Agricultural Research Center
FAO	Food and Agricultural Organization
FOC	*Fusarium oxysporum* f. sp. *Ciceris*
EIAR	Ethiopian Institution of Agricultural Research
IDM	Integrated Disease Management
LSD	Least Significance Difference
M.a.s.l	Meter above sea level
PSI	Percent severity index
SAS	Statistical Analysis System
SPSS	Statistical Packaging of Social Science

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Chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris)* intensity in North Shoa, Ethiopia, and Evaluation of Chickpea Genotypes against the pathogen

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ABSTRACT

Chickpea (Cicer arietinum L) is an important pulse crop in Ethiopia. However, chickpea Fusarium wilt (CFW) is the major constraint on its production. Thus, this study was designed with the following objectives: to assess the prevalence, incidence, and association of biophysical factors with CFW in North Shoa Ethiopia and evaluate chickpea genotypes against the pathogen. For this purpose, a field survey was conducted during the 2022 main growing season. Descriptive statistics and logistic regression model were used to analyze association of the disease with different biophysical factors. Fungal isolation was done out by placing small bits of infected root on PDA at Hawassa University Crop Protection Laboratory. Colony characteristic of the fungus were studied on PDA. The spores were obtained by growing the fungal pure culture on SNA. The pathogen was morphologically identified using appropriate identification manual. Sixteen chickpea genotypes were screened for their reaction to CFW under a lath house condition. The survey results revealed that CFW was prevalent in all the surveyed areas, with different levels of incidence. A higher CFW incidence (43.2%) was recorded at Siyadebrna-wayu district, while a lower (34.8%) CFW incidence was recorded at Ensaro district. High mean CFW incidence was recorded on chickpea sown late August (39.2), desi-type chickpea (39.7), fields previously sown with legumes (43.9),lower altitudes (40.3), pod setting stage (41.2) and weedy fields (45.8%). In the model, crop growth stage, district, previous crop, and weeding practice were significantly associated with FOC incidence. The highest (>38%) wilt incidence was highly associated with weedy fields. The fungal mycelia were white in color. The macro conidia were straight to slightly curve with three septa. While the micro conidia were oval and no septation, Based on these, the pathogen was identified as F. oxysporum f. sp. ciceris (FOC). Three chickpea genotypes (DZ-2012-CK-0312 followed by FLIP12-138c and ICCMABCD-21) were moderately resistant, while nine chickpea genotypes were susceptible. The result of the current study confirmed that CFW was highly prevalent in the study areas. Thus, designing effective CFW management options by targeting important biophysical factors that influence disease pressure and using chickpea varieties that have some degree of resistance to the disease is essential. It would be better to repeat the late house experiment for one or two times and further tests under field conditions to come up with sound recommendations.

Key words: Biophysical factor; Chickpea; CFW; Genotype; Incidence; Prevalence; Screening

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) originated in the Middle East (Southern Eastern Turkey and the surrounding areas of Syria) (Kujur *et al*., 2015) and belongs to the family Fabaceae or Leguminosae (Tehulie and Yimam, 2021). It is a self-pollinating legume crop with a diploid set of chromosomes (2n = 16) (Varshney *et al.,* 2013) cultivated for its edible seeds in almost all parts of the world. According to Rathore *et al*. (2021), chickpea seeds contain 22.1-24.42% protein, 0.15-1.25 sulfur-containing amino acids, and 0.63-1.38% tryptophan. In addition, it can fix atmospheric nitrogen through rhizobium bacteria present in their roots, which in turn improves soil fertility. Hence, the crops sown next to the chickpea also benefit. Due to the tap root system, it opens up the soil, and the extensive leaf drop increases the organic matter in the soil (Korbu *et al*., 2020).

Chickpea, also known as Bengal gram, garbanzo bean, and Shimbra, occupies more than 20% of the world's pulse production, only being exceeded by common beans and peas (Patra *et al*., 2017). India, Pakistan, Australia, Canada, Mexico, and Turkey are the world's major chickpea producers, and India is the leading producer (Deshmukh, 2005).

Ethiopia is a secondary center of diversity for chickpea (Arriagada *et al*., 2022), which grows in Ethiopia's northern, central, and eastern highlands (Woldekiros and Mekonnen, 2018; Sokolkova *et al*., 2020). The crop is the third most important pulse crop in terms of grain production after Faba bean and Haricot bean (CSA, 2021/22) in the country. In African countries, Ethiopia is the leading producer of chickpea and covers 46% of the continent's production (Ayana *et al.,* 2019). Furthermore, the Amhara and Oromia regions are the largest producers of chickpeas in the country (Tigabie *et al*., 2020). Also, the North Shoa Zone is one of the top chickpea producer zones in the Amhara region (Mekbib and Chane, 2011). Chickpeas contribute 12.66% of Ethiopia's total pulse production and cover about 0.24 million ha of the grain crop area (CSA, 2018/2021/22).

However, chickpea's productivity in Ethiopia remains as low as 2000 kg/ha (CSA, 2022), while the crop potential yield is up to 5000 kg/ha (Verkaart *et al*., 2017; Ayana *et al*., 2019). Both abiotic and biotic factors are responsible for the low productivity of crops. Some of the

abiotic factors are a low adoption rate of new chickpea technologies, a weak seed delivery system, a lack of adapted varieties suitable to growing conditions, poor crop management practices, and terminal drought (Woldekiros and Mekonnen, 2018; Yimer *et al*., 2018). And from biotic factors, plant diseases and insect pests are the major production problems for chickpeas. Different insect pests, such as African bollworm, cutworms, aphids, jassids, thrips, white flies, or storage bruchids, that have been the most destructive in Asia, Africa, and Australia, pose a serious challenge to chickpea production and productivity (Mihretie *et al*., 2020).

About 172 pathogens have been reported to affect chickpea world-wide, and from these pathogens, which lead to an overall reduction in chickpea, fungal diseases are of prime importance, followed by viral and bacterial diseases that affect all parts of the plant at all stages of growth (Pande *et al.,* 2012). Among fungal diseases, some soil-borne diseases are Fusarium wilt, dry root rot, wet root rot, black root rot, and collar rot caused by *Fusarium oxysporum f.sp. Ciceris, Rhizoctonia bataticola, Rhizoctonia solani, Fusarium solani, and Sclerotium rolfsii,* respectively (Mageshwara *et al*., 2022). In Ethiopia, the major fungal diseases of chickpeas are Fusarium wilt, Ascochyta blight, and dry root rot (Yimer *et al*., 2018).

Chickpea Fusarium wilt, which is one of the main root diseases of chickpea, is reported in about 32 countries, such as the Indian subcontinent, the United States, Mexico, Spain, Tunisia, Turkey, and Ethiopia, where the chickpea growing season is dry and warm, inflicting quantitative as well as qualitative losses (Sharma, 2019; Asrat and Tolosa, 2020). This pathogen can cause infection at all stages of plant growth, with more incidences in the flowering and podding stages (Maitlo *et al.,* 2014). Attacks from the Fusarium wilt pathogen can destroy a crop completely or cause significant annual yield losses. According to Endalew *et al*. (2022), the disease cause annual average yield loss of up to 10–90%, and in higher relative humidity and ambient temperature, it can cause 100% yield loss of chickpea. Due to Fusarium wilt, 10%, 40%, and 17% annual chickpea yield losses were estimated in India, Tunisia, and Iran, respectively, as described in Hotkar *et al. (2018) and* Karimi *et al*. (2012).

And also, about 30% of the yield loss of chickpeas due to this pathogen has been reported by Meki *et al*. (2008) in Ethiopia. Merkuz *et al*. (2011) also reported that chickpea Fusarium wilt incidence was in the range of 27.84% in Dejen district to 37.90% in Dembia district in Northwestern Ethiopia. Similarly, a survey conducted for two consecutive years during the 2013–14 and 2014–15 growing seasons found that wilt and root rot of chickpeas was prevalent in all surveyed areas of the country (Damte and Ojiewo, 2016). Moreover, Ali and Terefe (2021) conducted a survey on chickpea Fusarium wilt in North Shoa Amhara, Ethiopia. The result showed that the disease was prevalent in all the surveyed areas (Ensaro, Merhabete, Moretna-jiru, Menz-mama, and Mojana-wedera), with disease incidences of 50.19%, 47.71%, 43.06%, 37.39%, and 34.27%, respectively, and caused significant damage to the crop. Furthermore, in the Asrat (2017) review paper, 7–17% (average 11%) chickpea wilt incidence was recorded in Debre Berhan.

Debre Birhan Agricultural Research Center (DBARC), which is found in this study area zone, undertook chickpea scale-up activity and distributed Arerti and Kutaye improved varieties to farmers. However, chickpea fields were heavily devastated by wilt pathogens, especially Chickpea Fusarium wilt (DBARC, 2016; Ali and Terefe, 2021). Despite the fact that chickpea Fusarium wilt management has remained difficult due to its' both soil and seed-born nature and single control measures are not fully effective, many disease management practices have been developed in different parts of the world.

Since the pathogen is a monocyclic disease in which development is driven by the pathogen's primary inoculum, disease management should be targeted at the exclusion of the pathogen as well as reducing the amount and/or efficiency of the initial inoculum (Jendoubi *et al*., 2017). For such a goal, the use of certified seeds free from *F. oxysporum* f. sp. *ciceris*, sanitation and cropping practices, choice of sowing site and time that favor the crop to be more vigorous and resistant to the disease and disfavor the pathogen reproduction, disease establishment, and development are some available alternatives to managing chickpeas Fusarium wilt that would be applicable in the absence of a resistant variety (Jimenez-Diaz *et al*., 2015). However, that may not be sufficient because of the soil- and seed-born nature of the pathogen (Nikam *et al*., 2007; Jimenez-Fernandez *et al*., 2011).

Trials also showed the possibility to manage chickpea Fusarium wilt using bio-agents like Trichoderma spp. and Bacillus subtilis. Using Pseudomonas and Bacillus spp. as a bio agent increases fresh and dry weight of chickpea in both seed treatment and soil inoculation technique than untreated one (Karimi *et al*., 2012). However, their effectiveness is determined by environmental factors, pathogen race and the inocula level.

A scholar tried to test the effectiveness of fungicides like Apron Star, Mancozeb, Thiram, and some other fungicides to control FOC (Yigrem *et al*., 2018). However, as chickpea is a rain-fed crop and is grown under low input conditions, and the pathogen is soil-transmitted, continuous seed treatment with fungicides is not possible.

Above all, the use of resistant varieties is the most economical and effective approach to managing or eradicating the disease (Jendoubi *et al*., 2017). In the absence of resistant or tolerant varieties, it would be too difficult to manage the disease caused by soil-borne pathogens because of their complex physico-chemical properties, environmental conditions, and biological origin. Moreover, the use of resistant varieties and cultivars would enhance the efficacy of other disease control methods in an integrated management strategy.

Due to this reason, many sources of resistance to *Fusarium oxysporum* f. sp. *ciceris* have been reported based on either field observations during natural epidemics or artificial inoculation in green house conditions from all over chickpea-growing countries. However, host resistance does not persist, as varieties presumed to be wilt-resistant failed, either as a result of genetic breakdown or a change in the virulence of the pathogen (Khan *et al.,* 2010). Therefore, there is a continuous need to screen and develop new varieties using different breeding techniques against virulent strains to create variability and obtain sustainable yields. But in the study, there is no frequent development of resistant varieties, so adequate resistant chickpea varieties in this area are lacking.

On the other hand, conducting a comprehensive and periodic disease survey, which can help generate better information about types of the disease, its current distribution and economic importance, available and effective disease management practices, and pertinent biophysical factors associated with the disease, is required (Damote and Ojiewo, 2016; Ali and Terefe, 2021). Such information is important in developing effective disease management practices. In

Ethiopia, different scholars conducted disease surveys to determine the status of chickpea Fusarium wilt (Yimer *et al.*, 2018). However, very limited efforts were put into the study area of the Northern Shoa Zone, especially in Siyadebrna-wayu district, about the disease distribution, prevalence, economic importance, and intensity

Chickpea Fusarium wilt is a very yield-limiting factor in the study area. This is because farmers do not implement necessary practices to prevent losing the crop to the diseases, and their prevalence, distribution, and intensity are not well addressed, and adequate resistant variety sources are also not available. Therefore, the present study is proposed with the following objectives;

1.1. Objectives

1.1.1. General objective

- To improve the productivity of chickpea in North Shoa, Ethiopia through the use of appropriate chickpea Fusarium wilt management methods

1.1.2. Specific objectives

- To assess the prevalence and incidence of chickpea Fusarium wilt in Siyadebrna-wayu and Ensaro districts of North Shoa, Ethiopia
- To determine the association of chickpea Fusarium wilt with biophysical factors in Siyadebrna-wayu and Ensaro districts of North Shoa, Ethiopia
- To evaluate the reactions of some chickpea genotypes against Fusarium wilt under lath house conditions.

2. LITERATURE REVIEW

2.1. Origin and domestication of chickpea

Chickpea comprises 10 annual and 36 perennial species. Among the annual species, *Cicer arietinum* is the only domesticated and cultivated species worldwide (Toker *et al*., 2021), and its archaeological remains were found in the Middle East back to 7500–6800 BC (Gayacharana *et al*., 2020). This context suggested that Vavilov is the primary center of chickpea origin in Southeastern Turkey, Syria, and the Mediterranean region, whereas the secondary centers of origin are South Asia and Ethiopia (Van der Maeso, 1987; Arriagada *et al.*, 2022).

Recently, a comprehensive study based on whole-genome resequencing of 429 lines sampled from 45 countries suggests that the chickpea migration route occurred from the Mediterranean region to South Asia and then to East Africa and Central Asia in parallel. In addition, migration to America occurred from Central Asia or Eastern Africa rather than the Mediterranean basin alone (Varshney *et al*., 2019).

2.2. Types of chickpea

Chickpea has Desi and Kabuli types. The Desi type is relatively small angular seeds with various coloring and sometimes spotted whereas, the Kabuli type is larger seed sizes that are smoother and generally light colored (Merga and Haji, 2019). Both have different genetic background in disease resistance and important agronomic traits (Kassie *et al*., 2019). Yimer *et al*. (2018) and Bekele *et al*. (2021) explained that Desi-type chickpea seeds found in Ethiopia are often landraces without modern genetic improvements. However, Ethiopian Kabuli-type varieties are typically the product of organized crop improvement efforts, and their availability is more frequently associated with organized seed systems and the higher acceptance of new agricultural technologies. Therefore, Desi type chickpea are relatively susceptible to disease than Kabuli type. According to Getaneh *et al*. (2021), the Ethiopian chickpea production is predominately about 95% by Desi chickpea, however currently, the interest of farmers in producing the large seeded Kabuli varieties increasing due to domestic and export market.

2.3. Growth requirement of chickpea in Ethiopia

Ethiopia has got diverse agro-ecologies that render it suitable as one of the highly productive geographies for chickpea cultivation in the world (Korbu *et al*., 2020; Fikre *et al.*, 2018).Chickpea is usually grown on black Vertisol which is known for excess water and drainage problem during the main rainy period (June–August).As a result, farmers plant chickpea late in the season (September–October) commonly on residual moisture. Previously, mid-August was considered the appropriate sowing date, but due to the "belg" rainy season, chickpea cultivation was heavily impacted by root rot, and in order to avoid this problem, plant in mid-September. However, the later sowing date presents a new issue, due to the elevation of highlands, which is frost stress (Admas *et al*., 2021)

Chickpea is usually cultivated without application of fertilizers and herbicides. In addition Pesticides are applied on chickpea fields to control diseases or insects only when a specific disease or insect epidemic occurs in a specific location. It is weeded at least once throughout the production season and its harvesting is done by manual labor, either for green pod consumption or for dry seed from October to March (Fikre and Bekele, 2019).

The plant requires fertile soil with good drainage system and if any waterlogged conditions can severely damage the crop. Chickpeas generally grow on heavy black or red soils and require a soil pH of 6.4 to 7.9 They prefer soil with good residual soil moisture content. Inoculating chickpeas with *Rhizobium*, when planting first time in virgin sandy soils or in heavier soils can increase yield by 10-62% (Chongo and Gossen, 2001). In Ethiopia, chickpea is best adapted to the areas having vertisol which have good water holding capacity (Abdula, 2013).

Chickpea is a self-pollinated crop and usually grown as a rain-fed cool-weather crop or as a dry climate crop in semi-arid regions. The optimum daily temperature ranges from 18 to 29°C. Occurrence of frost and hailstones can severely damage the crop. Though sensitive to cold, some cultivars can tolerate temperatures as low as -9.5°C in early stages. A relative humidity of 21-41% is optimum for seed setting (Assfaw *et al.,* 2019).

The crop grows well in areas with annual rainfall of between 700 – 2000mm. However, chickpea productivity under marginal rainfall conditions may be increased through genotype

selection and manipulation of planting density. Owing to its deep tap root, chickpea is fairly drought tolerant as it is able to extract moisture from deep layers of soil profile, but its productivity is reduced by the recurrence of the terminal droughts (Abdula, 2013)

2.4. Production Constraints of Chickpea

2.4.1. Abiotic constraints

Chickpea production is exposed to different abiotic and biotic constrains which reduces seed yields. The most common abiotic stresses affecting chickpea production are, salinity, heat, frost, cold and drought (particularly, soil moisture deficit towards end of the crop season) and resistance and tolerance to these stresses is more complex (Guar *et al*., 2019; Mekonnen, 2020). As Kemal *et al*. (2018) reported, water logging during the main rainy season and terminal moisture stress when chickpea is grown at the end of the season using residual moisture were some of the major obstacles for chickpea production in vertisol areas of Ethiopia. On the other hand, the crop is sensitive to salinity stress, particularly during the early stages of growth and development since seedling growth is the most critical life stage of the plant (Zewude *et al*., 2017). The other abiotic factors to chickpea is frost stress which takes place late in the podding and flowering stages and during these stages causes issues such as flower abortion, poor pod set, and impaired pod filling, leading to a drastic reduction in yield and quality (Croser *et al*., 2003). Drought stress exacerbates an infection that is already present by decreasing the endodermal barrier and overall defense (Sachdeva *et al*., 2022).

2.4.2. Biotic constraints

The main biotic stresses which lead to yield reduction and insecurity are those caused by fungal, bacterial and viral diseases, insect pests, parasitic nematodes and parasitic weeds of chickpea. Some of the diseases caused by biotic stresses are described below. Ascochyta blight, caused by *Ascochyta rabiei*, is the most important yield- and quality-limiting foliar disease of chickpea reported from all chickpea growing areas (Mart *et al*., 2022). It occurs mainly in areas where cool, cloudy and humid weather prevails during the crop season (Gayacharan *et al*., 2020; Getaneh, *et al*., 2021). Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *ciceris*, is the most important root disease of chickpea, particularly in the semiarid tropics where the chickpea growing season is dry and warm(Sharma,2019;Zewdie &

Bedasa, 2020). Furthermore, chickpea dry root rot caused by *Macrophomina phaseolina* (previously *Rhizoctonia bataticola*) is a financially destructive disease that has harmed chickpea yield in recent years as a result of irregular rainfall patterns (Yimer *et al*., 2018; Mageshwaran *et al.*, 2022).

Viral diseases have been reported to cause sporadic but significant yield loss in some areas. Major symptoms include discoloring (yellow, orange or brown) of foliage, browning of phloem and stunting of growth (Vanitha *et al*., 2020). Many viruses have been identified that can cause stunt disease, from these viruses, chickpea stunt virus is considered the most important chickpea disease after fungal wilt and root rot disease(Abraham *et al*., 2006).

Production of chickpea also interrupt mainly by gram caterpillar or gram pod borer (*Helicoverpa armigera Hubner*) which is highly polyphagous and sources with high levels of resistance are not available in chickpea germplasm. As Damte and Mitiku (2020) and Fite and Tefera (2022) reported, chickpea production was limited due to Insect pests mainly *Helicoverpa armigera Hübner* is the major factors of chickpea cause up to 33% pod damage at field in Ethiopia. Furthermore, according to the same author, *Callosobruchus chinensis* is the most important storage pest of chickpea, cause up to50% weight losses in stored chickpea in Ethiopia.

Some nematode species are considered constraints to chickpea production, causing an estimated 14% in annual yield losses (Jimenez-Diaz *et al*., 2015). This includes three types of nematodes causing major economic damage to chickpea crops globally, namely, root-knot nematodes (*Meloidogyne artiella*, *M. incognita*, and *M. javanica*), chickpea cyst nematode (*Heterodera ciceris*) and root-lesion nematode (*Pratylenchus thornei*) (Zwart *et al*., 2019).

Weeds impose severe yield penalty depending upon intensity, time of emergence, duration of infestation and competing ability of crops (Nibhria *et al*., 2022). Hence, Chickpea, being slow in its early growth and short stature plant, is highly susceptible to weed competition and often considerable losses may occur if weeds are not controlled at proper time(Merga & Alemu, 2019). Generally, Cool-season broadleaf weeds are the most difficult to control in the chickpea since they have the same ecology and biology with the crop (Yenish, 2007).

2.2. Distribution of Chickpea Fusarium Wilt (CFW) and Yield Losses

Chickpea Fusarium wilt was first recognized in India by Butler in 1918 as indicated by Jimenez-diaz *et al*. (2015). It is reported in about 32 countries such as Indian sub-continent, the United States, Mexico, Spain, Tunisia, Turkey and Ethiopia, but it has not yet been reported in Australia(Cunnington *et al*.,2007;Sharma, 2019).This pathogen can cause infection at all stages of plant growth with more incidences in flowering and podding stage (Maitlo *et al*, 2014). Attacks from Fusarium wilt pathogen can destroy a crop completely or cause significant annual yield losses. According to Endalew *et al*. (2022), the disease causes annual average yield loss up to 10–90% and in higher relative humidity and ambient temperature, it can cause 100% yield loss of chickpea. Early wilting is reported to cause more yield loss than late wilting, however seeds from late-wilted plants are lighter, rougher, and duller than those from healthy plants (Jimenez-diaz *et al*., 2015). Due to Fusarium wilt 10%, 40% 17% Annual chickpea yield losses were estimated in India, Tunisia and Iran respectively as described in the (Hotkar *et al*.,2021; Karimi *et al*. 2012).

In Ethiopia, about 30% yield loss of chickpea due to chickpea wilt has been reported (Meki *et al*., 2008).Its distribution in North Gondar, South Gondar and East Gojjam administrative zones of north-western Ethiopia was reported during the 2006–2007 and 2007–2008 main crop seasons by Abera *et al.* (2011) and they reported that, it was found to be prevalent in almost all the surveyed chickpea-growing areas. In addition Damte and Ojiewo (2016) stated that the chickpea wilt distribution from low to high incidence in East Gojjam, South West Shewa, North Shewa and West Shewa in 2013/14 and 2014/15 was observed. Moreover, Yimer *et al*. (2018) report showed that, Mean percent wilt and root rot incidence and percent severity index were the highest in Gojam followed by Gondar and the lowest in Shoa.

Results of surveys conducted during the 1984/85 season showed up to 25% disease incidence in Ambo and Nazreth areas of Shewa Administrative regions (IAR, unpublished). The incidence of chickpea wilt ranged from 1% to 41% (Average 25%) in Gohatsion, 4-35% (average 17%) In Nazreth 3-31% (average 15%) in Wolkite, 1- 18% (average 11%) in Ambo, and 7-17% (average 11%) in Debre Berhan (Asrat,2017).

Fusarium causes vascular wilts of different annual and perennial plants. Species of *Fusarium oxysporum* are diseases causing fungi; disease is resulted by different strains of the species that has different host plants (Agrios, 2005; Leslie and Summerell, 2006). The fungus that attacks tomato is designated *F. oxysporum* f.sp. *lycopersici*; cucurbits, *F. oxysporum* f.sp. *conglutinans*; banana, *F. oxysporum* f.sp. *cubense*; cotton, *F. oxysporum* f.sp. *vasinfectum*; carnation, *F. oxysporum* f.sp. *dianthii*; and so on. Wilts occur as a result of the activities of the pathogen in the xylem vessels of the plant. In annual crops entire plants may die within weeks, whereas in perennials, death may not occur until several months or years after infection has been takes place. Unless the infected host plant is not dead, the pathogen remains in the xylem of the host. But, when the host plant is dead the pathogen starts sporulation at or near the surface of the dead host plant (Agrios, 2005).

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

According to Agrios (2005) the pathogen is classified as follows: Kingdom: *Mycota,* Division: *Eumycota,* Sub-Division: *Deuteromycotina,* Class: *Hyphomycetes,* Fungi Imperfect, Order: *Moniliales,* Family: *Tuberculariaceae,* Genus: *Fusarium* species: *Fusarium oxysporum* f.sp. *ciceris.* The genus Fusarium was introduced by Link in 1809, and is now approaching its third century as a genus that contains many plant-pathogenic fungi (Leslie and Summerell, 2000). Furthermore, the members of this genus can incite directly diseases in plants, humans, and domesticated animals. It contains over 20 species including *Fusarium oxysporum, Fusarium solani, Fusarium equiseti* and *Fusarium chlamydosporum* (Okugbowa and Shitu, 2012).

2.3.1. Isolation of Fusarium species

Several selective media have been developed for the isolation, growth and sporulation of Fusarium species, including Selective Fusarium Agar (SFA), Dichloran Chloramphenicol Peptone Agar (DCPA), Spezieller Nahrstoffarmer Agar (SNA) and Modified Potato Dextrose Agar (MPDA). The isolation of *Fusarium* species from plants is affected by the nature of the source material, method of surface sterilization, plating procedures, medium and incubation conditions (Burgess *et al.,* 1994). The choice of medium depends largely on the nature of the tissue involved in the isolation exercise. Selective media are normally used for the isolation of Fusarium species from diseased crown or root samples. There are several other techniques for

recovering Fusarium species, directly or indirectly, from plant samples, which do not involve plating tissue segments on agar media. Some species produce sporodochia on the surface of the diseased tissue. Macro conidia can be taken from these sites and used to prepare a conidial suspension, which is plated on Water Agar containing antibiotics. Germinated single conidia are later taken to initiate pure cultures for identification of Fusarium species (Burgess *et al.*, 1994).

2.3.1. Morphology of *Fusarium oxysporum*

Fusarium oxysporum is an asexual fungal species that includes human and animal pathogens and a diverse range of non-pathogens. Pathogenic and nonpathogenic strains of this species can be distinguished from each other with pathogenicity tests. No sexual stage is yet known for *F. oxysporum*. Morphological characteristics of the anamorph stage of the pathogen like presence and absence of micro conidia and chlamydospores, size and shape of micro- and macro conidia, colony color and texture, and conidiophores structure are very important during grouping of the pathogen and to determine its taxonomic position (Gordon, 2017). The aerial mycelium of *Fusarium oxysporum* f.sp. *ciceris* is first white and cottony, but later it may become cream or salmon in color or remain white on potato sucrose agar and potato dextrose agar (Jimenez Diaz *et al.*, 2011).

FOC produce micro conidia, macro conidia and chlamydospore. The micro conidias are oval or cylindrical, straight or curved, have 0-1 septa (usually 0) and occurs on short micro conidiophores. Macro conidias are produced more sparsely than micro conidias and they are thin walled and 2-5 septa (usually 3) or fusoid. Chlamydospores are formed in 15-day-old cultures in hyphae or conidia and infected chickpea tissues. They may be formed singly, in pairs or in chains, and are smooth or rough in their structure (Leslie and summerell ,2000; Jimenez Diaz *et al.*, 2011; Castro *et al*., 2012). The pathogen also possesses branched and septated hyphae (Jimenez Diaz *et al*., 2011).

2.3.2. Ecology and epidemiology of the pathogen

FOC is both seed and soil borne, saprophytic fungus optional in absence of its host, and it can survive in the soil and crop debris by means of chlamydospores at least for 6 years, but it can also survive good in infected plant roots and stems (Chudasama and Pithia, 2018). However

infection of symptomless dicotyledonous weeds can enhance survival of the pathogen in fallow soils. This indicates, infested soil is a main source of primary inoculum for the development of Fusarium wilt epidemics in chickpea (Patra *et al*., 2017). It is difficult to check the disease or eliminate the pathogen, when the inoculum is developed in the soil except by following crop rotation for more than six years (Roy *et al*., 2017).

The conidia of the pathogen are short lived. Chlamydospores are released into the soil when the crop debris is decomposed and incorporated in to the soil (Haware and Nene 1982). The primary infection may be through Chlamydospores or mycelia of the organism. The fungus can survive in adverse conditions of temperature and pH. Even if the fungus can grow at temperatures as low as 7℃, and as high as 35℃, and a pH value ranging from 4-9.4, the optimum temperature for good mycelial growth is 25-27℃, and a pH value of 7.1–7.9 (JimenezDiaz *et al*., 2011; Rafiq *et al*., 2020). The yellowing pathotype can grow at a higher rate than the wilting pathotype under the same temperature (DuroAlmazan, 2000; Sunkad *et al.*, 2019).

2.3.3. Pathogen Variability

FOC have extreme genotypic and phenotypic variability and can adapt to a wide range of environmental conditions. Two pathotypes distinguished based on distinct yellowing or wilting syndrome that induce in susceptible chickpeas and Eight races of the pathogen have been identified worldwide using standard set of differentials (Jimenez *et al*., 2015).The yellowing syndrome is characterized by slow, progressive foliar yellowing and late death of the plant, while the wilting syndrome is characterized by fast and severe chlorosis flaccidity and early plant death.

The eight *F. oxysporum* f. sp. *ciceris* races (race 0, 1A, 1B/C, 2, 3, 4, 5 and 6) also differ in their pathotype and geographic distribution. Races 1A, 2, 3, and 4 have been reported from India, and races 0, 1A, 1B/C, 5, and 6, from the United States and Spain (Sharma *et al*., 2012). Races 0 and 1B/C belong to the yellowing pathotype whereas races 1A, 2, 3, 4,5and 6 belong to the wilting pathotype. According to Meki *et al.* (2008) races 2, 3, and 4 are reported in Ethiopia from the identified pathogen races 0, 2, 3 and 4.

2.3.4. Infection process and 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). As Jimenez-Daiz *et al*. (2015), Lodhi *et al*. (2006) and Rafiq *et al*. (2020) described, first the fungus attacks the root apices or wounded root parts and penetrates to epidermis, cortex and colonize in vascular bundle and also penetrate to adjacent plant tissues. Then dense gels and histological distortion of xylem occurs in the vascular bundle and plug the normal flow of water and nutrients in the xylem vessel causes wilting, drooping, yellowing of leaves occurs and eventually whole plant is collapsed in few days. Finally, it remains in soil, roots, seeds and plant residues as chlamydospores.

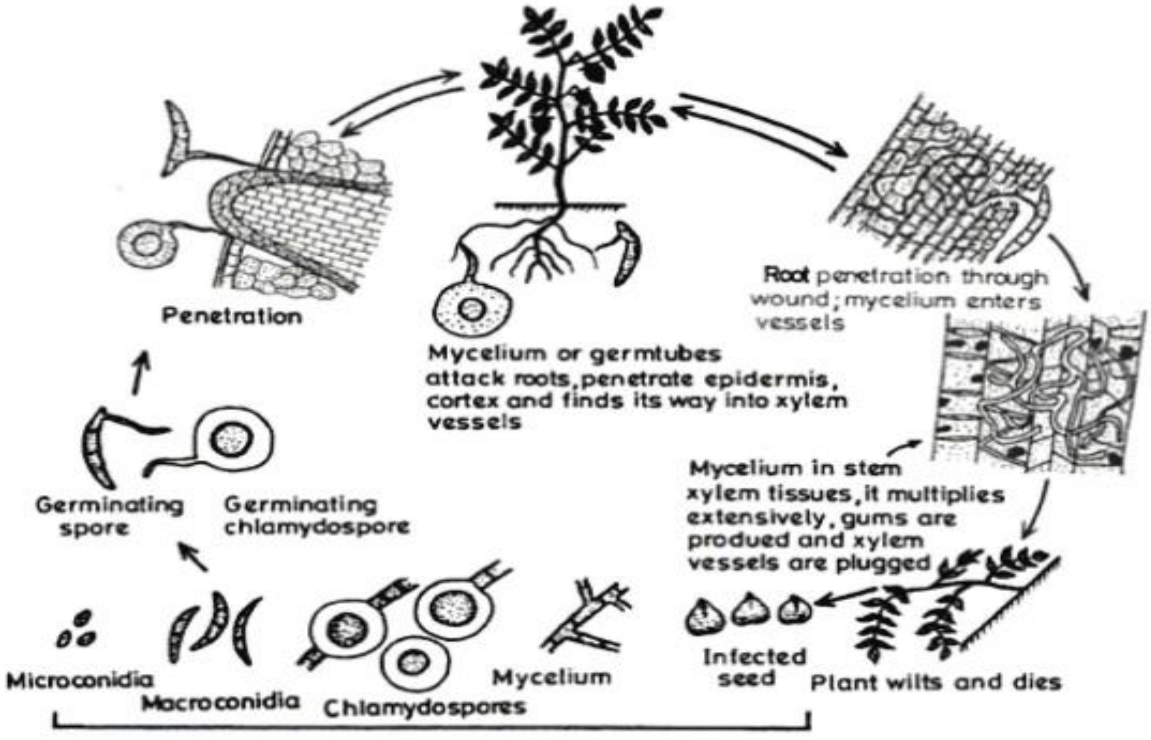

Figure 1. Life cycle of *Fusarium oxysporum* f.sp.*ciceris* (Lodhi *et al*., 2006; Rafiq *et al*., (2020).

Fusarium wilt 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. It's chlamydospores germination being stimulated by seed and root exudates of hosts and non-hosts. FOC gains ingress in germinating seeds and growing seedlings directly without need of wounds soon after sowing in infested soil. The invasion takes place mainly through the cotyledons and zones of the epicotyls and hypocotyls at the junction of or close to cotyledons, and to a lesser extent in the zone of root elongation and maturation (Jimenez- Díaz *et al*., (1989); Stevenson *et al.* (1997).

Later studies of Jime'nez'Ferna'ndez *et al*. (2013) in infested hydroponic cultures showed that races 0 and 5 of the pathogen invade the surface of the tap and lateral roots in both susceptible and resistant cultivars, and preferentially penetrate the meristematic cells of the root ape. Then, the fungus grows in the intercellular spaces of the root cortex to reach the central root cylinder and enter into the xylem vessels. Further colonization by the pathogen takes place through hyphal growth and micro conidia carried in the vessels by transpiration stream, as well as by lateral mycelia spread to adjacent vessels (Jimenez-Diaz *et al*., 2015). The systemic colonization along the plant axis (i.e., the determinative phase of pathogenesis) is then followed by development of symptoms (i.e., the expressive phase) once intense colonization of xylem vessels in root and lower stem has occurred by 10-20 days after inoculation(Jimenez-Diaz *et al.,* 2015).

2.3.5. Symptoms of Chickpea Fusarium wilt

Fusarium oxysporum f. sp. ciceris, is a soil or seed borne root pathogens that colonizes and proliferates in the vascular tissue disrupting the translocation of water thereby causing typical wilt symptoms (Taxak *et al*.,2017),Which include wilting, necrosis, damping off, withering, drooping, dull-green discoloration, yellowing, loss of turgidity in leaves, browning of vascular system and eventual collapse of whole plant and the wilted plants may be speared across the field or grouped in patches (Rafiq *et al*.,2020). Symptoms of the disease can develop at any stage of plant growth. However symptoms appear in different time duration as cultivars susceptibility. Thus, highly susceptible cultivars can show symptoms within 25 days after sowing and termed as early wilt, whereas, plants show symptoms within 6-8 weeks after sowing termed as late wilt (Rafiq *et al*., 2020).

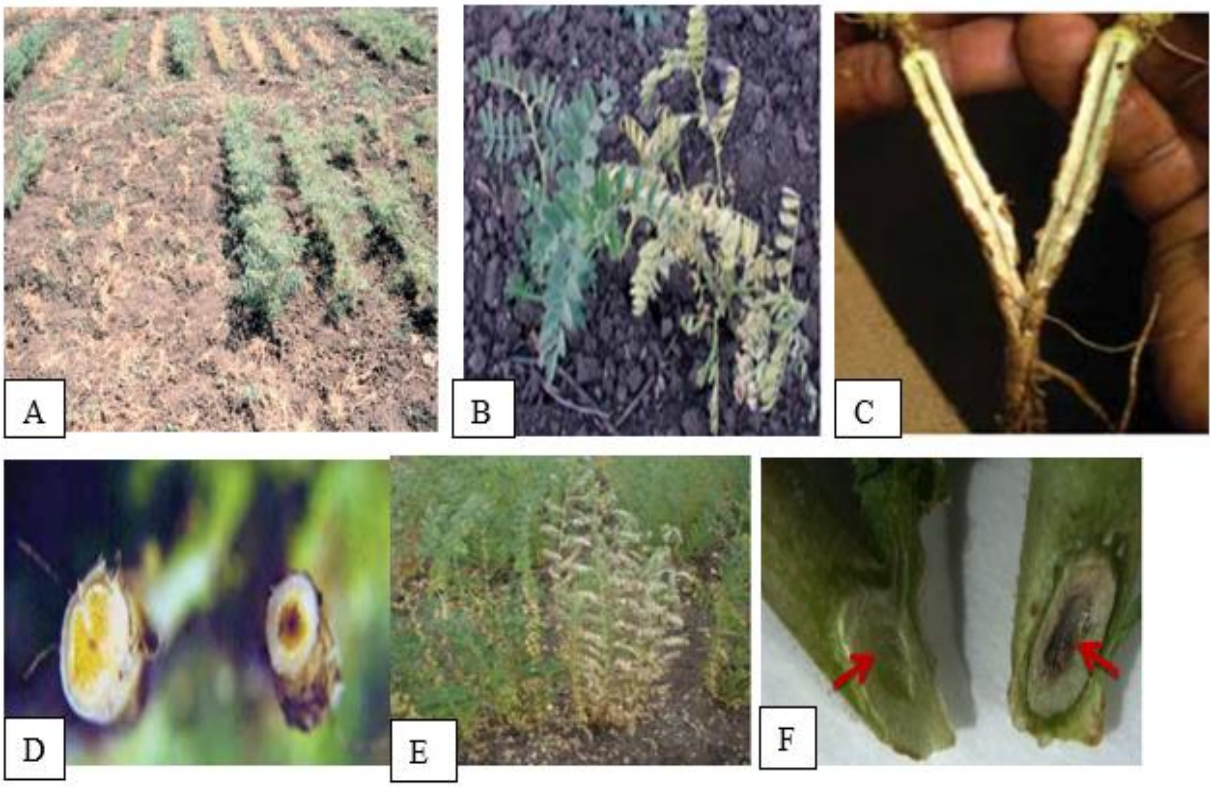

Figure 2. Symptoms of chickpea Fusarium wilt. A= Chickpeas affected by Fusarium wilt, B= Young chickpea plant with dull green leaves killed by Fusarium wilt, C Internal blackening of the stem caused by Fusarium wilt, D = A transverse cut of stem showing xylem blackening, caused by Fusarium wilt of chickpea, E = Drooping of Fusarium wilt-affected plant, F = Stem discoloration of a chickpea plant artificially infected by FOC. Healthy plant on the left (Nene *et al.*, 2012; Jendoubi *et al.*, 2012).

2.3.6. Host range

This pathogen is pathogenic solely on *Cicer spp*. From which, chickpea is the only cultivated species. However, *F. oxysporum* f. sp. *ciceris* can also invade root tissues of other grain legumes such as Faba bean (*Vicia faba*), Lentil (*Lens culinaris*), Field pea (*Pisum sativum*), and Pigeon pea (*Cajanus cajans*) without causing external symptoms, thus serving as symptomless carriers of the pathogen. Other crops and dicotyledonous weeds such as Cyperus rotendus, Tribulus terrestris, Convolvulus arvensis and Cardiospermum halicacabum (Nene *et al*., 1980) also serve as symptomless carriers (Jimenez-Diaz *et al*., 2015; Cunnington *et al*., 2016 and Gordon, 2017).

2.3.7. Disease Spread mechanisms

In chickpea, *F. oxysporum* f. sp. *ciceris* can be internally seed-borne and the pathogen is found as chlamydospore like structures in the helium region of the seed. Thus, the movement of infected seed plays an important role in the long distance dispersal and introduction of *Fusarium* wilt diseases into new areas (Jimenez-Diaz-2015). Whereas, infested soil and/or chickpea debris through human activity, machinery and water and can carry chlamydospores and conidia to spread the pathogen in short distances to surrounding plants and adjoining paddocks (Pande *et al*., 2007). However, windblown infected crop debris transport pathogen inoculums to a moderate distance (Gordon, 2017).

2.4. Management of Chickpea Fusarium Wilt

The management of chickpea Fusarium wilt should be targeted to exclusion of the pathogen as well as reducing the amount and/or efficiency of the initial inoculum since it is a monocyclic disease (Jimenez-Diaz *et al*., 2015). It can be done through host resistance, cultural practices, biological control, chemical protection and integrated management. 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. Some management methods to Fusarium wilt is present below.

2.4.1. Host Resistance

The use of resistant varieties is widely recognized as the safest, most economical and effective crop protection method to control soil-borne diseases (Pande *et al.*, 2020). 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). In Pakistan, Ahmed *et al.* (2010) tested 321 genotypes for Fusarium wilt resistance at seedling and reproductive stage and found 82 resistant genotypes at both stages. Similar studies were made by Khalifa *et al*., (2002) reported that, out of 41 evaluated genotypes 5, 16 , 18 and 2 genotypes were resistant, moderately resistant, susceptible and highly susceptible respectively in Algeria.

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 chickpea 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. At Adet Agricultural research center naturally on the field condition and artificially in screen house test, Desi and Kabuli chickpea varieties were evaluate against Fusarium wilt and both of them were not highly resistant as Ayana *et al.* (2019) reported. Whereas, Assfaw *et al*. (2019) showed that, resistance of Arerti and Shasho, moderately resistance of Habru and susceptibility of local Dz-10-4 of chickpea varieties for the identified Fusarium wilt of chickpea.

However, resistant cultivars of chickpea have not been utilized extensively due to the presence of undesirable agronomic characteristics in some developed materials and occurrence of higher pathogenic variability in the FOC population (Bayraktar and Dolar, 2012). As reported by Jimenez-Gasco *et al*. (2004) resistant varieties may sometimes be useful against certain FOC races.

2.4.2. Cultural practices

Fusarium wilt can be managed through the use of disease prevention strategies, such as rotation with non-host crop species, control of volunteer pulse crop plants in cereal crops, and control of annual weeds in crop borders and headlands. These practices helped in reducing the pathogen population in the soil but do not eliminate it completely (Agrios, 2005). Temperature, nutrient and pH of soil determine the virulence nature of Fusarium wilt. Subsequently influences the amount of yield loss that could be recorded due to the disease. 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. According to Chand and Khirbat (2011) study, the amount of organic matter is inversely related to wilt incidence and delay in sowing helps in minimizing disease. Similarly, effects of altering dates of sowing on the incidence of chickpea wilt were assessed at DZARC (Asrat, 2017) and the recovery of wilt causing pathogen was decreased with delayed sowings. Abera *et al.* (2011) reported that, Fusarium wilt incidence was reduced with different doses of green manure and dried plant residue, but none of the treatments showed

complete disease suppression. Singh *et al.* (2006) noted that, wheat, barley, linseed and mustard intercrops/mixed cropping with chickpea were reduced wilt incidence and increased chickpea yield.

On the other hand, Use of clean seed for sowing and/or the use of fungicidal seed treatments can eliminate or reduce contaminating inoculum sources. Avoiding growing of chickpea crop in fields those are infected by the pathogen before planting the crop is also the other possible option to control disease risk and maximize the use of pathogen free seeds (Jimenez Gasco, 2011). Soil solarization with polyethene sheet for 6-8 weeks increase chickpea yield by reducing initial inoculum below threshold level and the process weakens the pathogen and reduces its virulence to the host crop, and increase its susceptibility to be attacked by other soil microbes (Strange, 2003).

2.4.3. Biological Control

The application of bio control agents to the soil is an alternative to suppress soil borne plant diseases through parasitism, production of antagonistic chemicals, competition for the host and nutrients, and induction of resistance in plants against disease-causing pathogens. As soil comprises a full ecosystem including many fungi, bacteria, insects, nematodes and other microbes, biological control is very important to understand those interactions to develop a soil health management strategy instead of focusing on individual disease causing species (Panth *et al.*, 2020).

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 under green house and field condition (Etebarian, 2006; Millan *et al*., 2006). These antagonists are saprophytic filamentous fungi, easily growing and produce conidia having long survival period in large quantities (Mohamed and Haggag, 2006). A research conducted by Dubey *et al.* (2007) revealed *T.viride* and *T. harzianum* could highly inhibit mycelia growth of *F. oxsporum* f.sp. *ciceris* and increases seed germination, and root and shoot length of the chickpea. A research result indicates that *Trichoderma viride* and *Trichoderma koningii* significantly restricts pathogen growth, but *Trichoderma harzianum* and *Gliocladium virens* shows moderate inhibition against the pathogen as Patil *et al.* (2015) research result indicated.

Bacteria also have bio-control potential for *Fusarium* wilt management. In Spain Hervas *et al.*, (1998) chickpea field soil treatment with *Pseudomonas fluorescens* showed the best *F.oxysporum* f. sp. *ciceris* suppression.

Non-pathogenic *Fusarium oxysporum* are also considered compatible biocontrol agents. They can suppress disease development by the pathogenic FO, competing for space and nutrients and even inducing resistance (Tropics, 1995). Likewise, Benhamou and garand, (2001) reported that, pea inoculation with a tomato non-pathogenic FO strain revealed an early stimulation of the defense responses on roots. Furthermore, the use of a non-pathogenic FOC isolate reduced the disease by 18% under controlled conditions in chickpea that indicated by Hervas *et al.* (1998).

2.4.4. Chemical Control

Under high inoculum density, suitable seed dressing fungicides are also most effective besides biocontrol agents (Garkoti *et al*., 2013). Chemical control of soil borne plant pathogens is generally preferred in large crop production areas due to relatively rapid effect and easy operation. Vitavax, thiram, bavistin, cabindazim, benomyl and captan fungicides were known by their role in reducing effect of chickpea Fusarium wilt (Christian *et al*., 2007).

For chickpea obtaining useful levels of *Fusarium* wilt control, seed-applied fungicides can be considered effective. A research conducted by Muhammad *et al*. (2011) to test the efficacy of fungicides results 100, 95.81, 93.80 and 70.96% reduction in mycelia growth at 5 ppm concentration of Derosal, Benomyl, Vitavax and Cabrio respectively. In an experiment conducted by Suman and Mohan, (2016) Carbendazim exhibited 100% fungal growth inhibition in both in-vivo and in-vitro conditions. Treating chickpea seeds with 0.15% Thiram + 0.1% carbendazim show effective management against FOC as reported by Nikam *et al*. (2007). And also Mohamed *et al.* (2016) demonstrated that, Thiram at100 ppm concentration level inhibited 95% of mycelial growth of the fungus in Sudan the fungicides.

2.4.5. Integrated Disease Management

Integrated disease management is a holistic approach that combines different disease management methods in an economically and ecologically-sound manner (Agrawal *et al*., 2002) Since the management of Fusarium wilt disease is a difficult task, not only in chickpea,

but in every plant species and relies on the integrated disease management approaches. Thus different researchers combined available disease management technologies to control chickpea fusarium wilt.

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 An experiment was conducted to test the effectiveness of integrating sowing date with chickpea cultivars that have good resistance level against CFW, and the research outcome shows that sowing chickpea in January together with resistant genotypes can reduce chickpea yield gaps (Seid *et al*., 2015) in Syria. A related result was found by Merkuz and Getachew, (2012) who integrated resistant cultivar, optimum sowing date and a raised seed bed and reduced chickpea wilt incidence. Zewdie (2019) also showed that; the lowest Fusarium wilt incidence (1.5%) was found on variety Arerti by raised bed and fungicide treated seeds.

On the other hand, integrating microorganisms like *Trichoderma harzianum*, *Pseudomonas fluorescens* antagonists and Rhizobacterium with fungicide suppresses FOC (Dubey *et al.*, 2015). Whereas, Hossain *et al.*, (2013) reported that the integration of soil treatment with *T.harzianum* isolate T-75 and *Az. indica* leaf extract and seed treatment with Provax-200 appeared to be significantly superior in reducing Fusarium wilt and in improving seed yield of chickpea compared to any single or dual application of them in the field.

3. MATERIALS AND METHODS

3.1. Description of Study Area

The field survey of chickpea wilt disease was conducted in two major chickpea growing districts (Siyadebrna-wayu and Ensaro) of North Shoa, Ethiopia (Figure 3). The survey area was purposively selected based on chickpea production potential and the frequent report of the wilt disease. The two districts are among the potential chickpea producing districts in the zone, according to zonal agricultural office report. Black soil is a common characteristic features for the two surveyed districts. Different climatic and geographical data of the surveyed districts were presented in Table 1.

Table 1. Description of the survey area

Districts	Altitude (m.a.s.l.)	Latitude (N)	Longitude (E)	Annual rainfall (mm)	Annual temperature(°C)
Siyadebrna – wayu	1900-2650	9°45’N	39°11’E	900-1000	10-22
Ensaro	1345-2663	9^{o} 48'N	38^{o} 54'E	900-1500	18-30

Source: District agricultural office record file (2022)

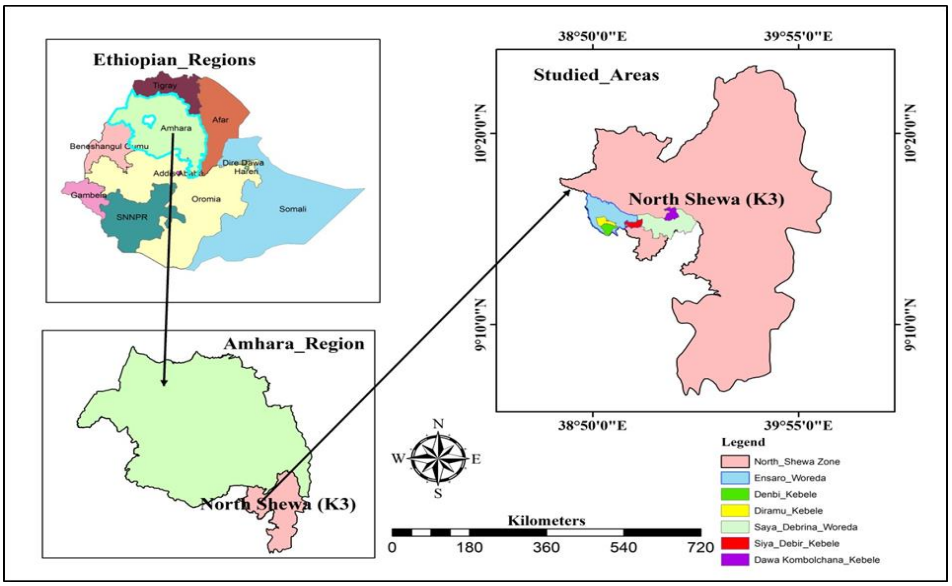

Figure 3. Map of Chickpea Fusarium wilt surveyed areas.

3.2. Survey Sampling and sampling procedure

Siyadebrna-wayu and Ensaro districts of the North Shoa Zone were purposively selected to conduct the survey in 2022 G.C. In addition, two kebeles were selected purposively from each district and 10 chickpea fields were randomly surveyed from each kebele. The field was assessed following main and feeder roads along 5-7 km. . In each chickpea field, disease assessments were conducted by dropping a 1 m^2 quadrat at five spots in an ‘’X” fashion based on the disease distribution in the field. A total of 40 chickpea fields were inspected during the survey. Formal survey questionnaires were prepared to capture data from farmers through personal interviews

3.3. Survey data collection

Infected fields were assessed for percent disease prevalence and it was calculated by the formula 1(Formula 1). In each quadrat the total number of plants and the number of plants showing various stages of chickpea wilt disease symptoms were counted in situ. Percent disease incidence was then calculated based on the formula 2 (Formula 2). The means of the five quadrats incidence were calculated for each field. 4 and 20 infected chickpea plants were collected in a separate paper bag from each quadrat and field respectively. In case of samples which included the root system with soil attached, the sample was put into a plastic bag and tied at the base of the stem (Plant Health Australia, 2008). All the samples were labeled with

the required information like the collection date, collection area, chickpea type, and sample code and brought to Hawassa University College of Agriculture Crop Protection laboratory for further isolation and identification purpose.

$$Prevalence\ (\%) = \frac{Number\ of\ fields\ \ infected\ by\ the\ disease}{Total\ number\ of\ fields\ assessed} x100 \ldots\ldots\ldots\ldots\ldots\text{Formula 1}$$

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ plants\ per\ quadrat}{Total\ number\ of\ plants\ assessed\ per\ quadrat} x100 \ldots\ldots\ldots\ldots\text{Formula 2}$$

During the survey both primary and secondary data were collected. The primary data was obtained through direct observation and personal interview. The secondary data was collected from reports from agricultural office of the selected districts. In addition to the disease data, the altitude of the field was recorded using a GPS device. Chickpea type, crop growth stage, weeding practices, soil type, previous crop history, cropping system, planting methods, and disease management practices were collected.

3.4. Pathogen isolation and identification

The wilt-affected chickpea plants were identified in the field based on key symptoms of chickpea Fusarium wilt, and 40 representative diseased chickpea samples were used for the isolation and identification of the chickpea wilt pathogen. The infected root and stem of the sample plants were cut into 0.5-cm pieces using sterilized scissors and washed under running tap water. The segments were surface disinfected with 70% ethanol for three minutes. The pieces were washed three times using sterilized distilled water and dried on sterilized filter paper. The pieces were then aseptically placed on a sterilized Petri dish containing Potato Dextrose Agar (PDA) and incubated at 25°C for 3 to 4 days (Lee *et al.*, 2007) for the recovery of the pathogens. Sub-culturing on the new PDA was done until pure culture was obtained. After pure cultures were obtained, they were stored at 4 ^{o}C for further studies.

The colony diameter of the pure cultures of the FOC isolates grown on PDA for 72 hours. Under dark conditions was measured by taking two radial measurements at a perpendicular angle and averaged. Observations on colony morphology and pigmentation were recorded after 7 days of inoculation on PDA medium by observing the surface and reverse sides of cultured plates, respectively.

The macro and micro-conidia were obtained by growing the fungal pure cultures on special media, i.e., Spezieller Nährstoffarmer Agar (SNA), under fluorescent light conditions for 10–14 days at the Hawassa University College of agriculture, crop protection laboratory. For this purpose, SNA was prepared by autoclaving in 1 L of distilled H_2O: 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, and 20 g agar. After getting the conidia, fungal identification was carried out by observing the macroconidia and microconidia under a compound microscope at 40x magnification. Then, using conidia structures, fungal cultures showing typical culture characteristics of *Fusarium oxysporuim* f.sp. *ciceris* were confirmed using morphological identification keys (Nelson *et al*., 1994; Leslie & Summerell, 2000; Burgess *et al*., 1994).

3.5. Pathogenicity test

The purified isolates were screened for their pathogenicity on a susceptible desi type chickpea variety (JG-62) under lath house conditions at Debre Zeit Agricultural Research Center (DZARC). The spore multiplication medium was prepared according to the method of Pande *et al.* (2012) with some modifications. Sand-maize meal medium was prepared by placing 180 g of sand, 20g of maize meal, and 40 ml of distilled water in bottles and autoclaving at 121 ^oC for 20 minutes at DZARC, plant pathology laboratory. The sterilized bottles, after cooling, were inoculated with four (0.8 mm diameter) discs of the pathogen isolates using a sterilized cork borer for inoculum mass production of the isolates. After plugging, these bottles were shaken for the first four days and incubated at 25 °C for 15 days (Appendix figure figure A).

Following these, a fungus-soil mixture was prepared by hand mixing the contents of each bottle with 4 kg of the sterilized (sterilized with an electronic sterilized machine for 2 hours) vertisol and sand mixture at a ratio of 3:1 under hygienic conditions (Appendix figure B) and filling it into a 30x20 cm plastic pot that was disinfected with sodium hypochlorite and washed with sterilized distilled water. Un-inoculated pots were used as a control (pots containing sterilized growth medium without fungal inoculum). Three pots were used as replicates for each isolate. The treatments were arranged using a completely randomized design (CRD) in the lath house. All the pots were given a small amount of water and kept for one week before sowing the chickpea seeds to allow the pathogens to establish themselves in the infested soil. Then, susceptible chickpea varieties (JG-62) were disinfected with 70%

ethanol for 3 minutes, washed with sterilized distilled water three times, and allowed to dry. Finally, 10 seeds per pot were sown into the soil mix at a depth of 3 cm. Adequate and regular watering was performed as necessary. After 15 days of sowing, the plants were observed regularly for the appearance and development of disease symptoms. As the symptoms of the disease appeared, the fungus was re-isolated from the roots of the diseased plant. The re-isolated fungus was transferred to PDA and SNA and compared morphologically with the original one using the same Fusarium identification manual that was used in the first step of identification under Section 3.3.

3.6. Evaluation of chickpea genotypes against chickpea Fusarium wilt

Mass production of Fusarium oxysporum f.sp. *ciceris*

For mass production of FOC used for screening of genotypes, sand maize meal medium was prepared in a 250-ml Erlenmeyer flask with contents of 90 g of sand, 10g of maize meal, and 20 ml of sterilized distilled water and autoclaved at 121 ^{o}C for 20 minutes. The sterilized flasks, after cooling, were inoculated with two disks (0.8 cm in diameter) of 7-day-old highly pathogenic FOC culture(Appendix figure 5A) using a sterilized cork borer and incubated at 25 °C for 15 days (Appendix figure 5B).

Experimental Treatments and Materials

The evaluations of chickpea genotypes against Fusarium wilt were conducted at Debrezeit Agricultural Research Center during 2022–2023 under lath house conditions. A total of 14 chickpea genotypes and 2 chickpea varieties (checks) were obtained from Debrezeit Agricultural Research Center (DZARC) (Table 2). Dhera and JG-62 were used as resistant and susceptible checks, respectively. Vertisol soil and sand at a ratio of 3:1 were sterilized for 2 hours by using an electric soil sterilizer machine, and the potting medium was prepared according to the method adopted by Pande *et al*. (2012) for the screening of these genotypes. The design used for the experiment was a completely randomized design (CRD) with three replications.

Table 2. Description of chickpea genotypes used for the screening experiment

S.No	Genotypes	Chickpea type	Remark
1	FLIP-13-344c	Kabuli	
2	DZ-2012-CK-2074	Kabuli	
3	FLIP-342c	Kabuli	
4	FLIP-88-85c	Kabuli	
5	DZ-2012-CK-0027	Desi	
6	FLIP13-379c	Kabuli	
7	DZ-2012-CK-0280	Kabuli	
8	DZ-2012-CK-0312	Desi	
9	FLIP09-262c	Kabuli	
10	ICCMABCD-21	Desi	
11	ICCU-16107	Desi	
12	FLIP12-138c	Kabuli	
13	ICCU-10	Desi	
14	FLIP12-07c	Kabuli	
15	JG-62	Desi	Susceptible check
16	DHERA	Kabuli	Resistant check

A fungus-soil mixture was prepared by hand mixing the contents of each flask with 2 kg of sterilized vertisol soil and sand mixture under hygienic conditions and filling them into sterilized plastic pots (15 cm in diameter). And also, un- inoculated pots (pots containing vertisol-sand mixture without inoculum of FOC) were prepared and used as control. All the pots were given little amount of water and kept for one week before sowing the seeds. After that, susceptible variety JG-62 were sterilized with 70% ethanol for three minutes, washed with sterilized distilled water three times, allowed to dry on filter paper and sown at a rate of 5 seeds per pot at the depth of 3cm. Adequate and regular watering was performed as necessary. After 90% of plants were wilted, healthy plants were removed and the wilted plants were chopped and incorporated into the potting medium. These pots were used for screening of the test genotypes against the FOC.

These pots were planted with the 14 test genotypes and 2 check varieties (Dhera and JG-62) separately. The seeds of the 14 test genotypes and 2 check varieties (Dhera and JG-62) were surface sterilized with 70% Ethanol alcohol for three minutes and washed with sterilized distilled water three times and allowed to dry on filter paper. Then 5 seeds per pot were sown at the depth of 3cm and adequate and regular watering was performed as necessary. The pots were arranged in the lath house using completely randomized design (CRD) with three replications. The un-inoculated pots (potting medium without FOC inoculum) were used as control. Germination was counted 10 days after sowing and the plants were observed regularly for the appearance and development of disease symptoms.

3.6.1. Data collection

3.6.1.1. Assessment of wilt incidence

The assessment of disease incidence was started three weeks after emergence, and the total number of wilted/ infected plants was recorded until the crop approached near physiological maturity. Disease incidence was recorded five times every 15 days, starting from the first appearance of disease symptoms. Plants that showed partial and complete wilt symptoms were considered wilted and tagged to avoid double counting in subsequent assessments. The wilt incidence was then calculated using the formula below.

$$\textbf{Wilt incidence } (\%) = \frac{Number\ of\ wilted\ plants}{Total\ number\ of\ plants/pot?} * 100$$

Based on the disease incidence data, the genotypes and varieties were categorized into different disease reaction groups, as indicated in Table 3.

Table 2. Reaction of chickpea genotypes to Fusarium wilt under lath house conditions

Grade	Disease incidence (%)	Disease reaction
1	0-10	Resistant
2	10.5-20	Moderately resistant
3	20.1-40	Moderately susceptible
4	40.1-100	Susceptible

Source; Pande *et al*. (2012)

3.7. Data analysis

3.7.1. Survey data analysis

Descriptive statistics were applied to determine the intensity of CFW in the study area. The association of CFW incidence with biophysical factors was determined by the logistic regression model using the statistical package for the social sciences (SPSS) version 26. Disease incidence was classified into distinct groups of binomial qualitative data, as described by Yuen, (2006). Based on the approximate similarity of the variables to the total assessed fields, class boundaries were estimated and the cut point was determined. Thus, ≤38 and >38 boundaries were selected for disease incidence. A cross tab of dependent and independent variable was constructed to represent the bivariate distribution of the fields (Table 4). First, the association of all the independent variables was tested on CFW incidence in a single-variable model. Second, the association of independent variables with the incidence of the disease was tested when entered first and last with all the other variables in the model. The odds ratio was obtained by exponentiation of the parameter estimates to compare the effect based on a reference point, which is interpreted as the relative risks of a specific factor for a specific disease (Yuen *et al*., 2006).

Table 3. Cross tab of variables used in logistic regression analysis

Independent variables	Variable class	No of field	CFW Incidence (%)	
			≤38	>38
Altitude	<2300	11	1	10
	>2300	29	16	13
Chickpea type	Kabuli	13	7	6
	Desi	27	10	17
District	S/wayu	20	5	15
	Ensaro	20	12	8
Growth stage	Vegetative	4	3	1
	Flowering	21	9	12
	Flower/pod setting	15	5	10
Planting date	August	28	11	17
	Early Sept	12	6	6
Previous crop	Cereals	26	14	11
	Legumes	14	3	11
Weeding practice	Weeded	26	15	11
	Weedy	14	2	12

3.7.2. Lath house experiment data analysis

An analysis of variance (ANOVA) using a SAS computer software package, version 9.0 (SAS, 2002) was performed for the disease incidence data of the screening experiment. Least significant difference (LSD) at 5% level of significance was used for mean separation.

4. RESULTS AND DISCUSSIONS

4.1. Field survey

4.1.1. General characteristics of the assessed chickpea fields

The altitude of the surveyed areas ranged from 2100 to 2650 m.a.s.l. Both Desi and Kabuli chickpea types were grown in the area. About 27 fields (67.5% of the total fields assessed) were planted with Desi-type chickpeas. The dominant varieties that grew in the surveyed areas were Kutaye, Natoli, Habru, Arerti, and Mastewal. The farmers locally call the disease "Leblib." None of the chickpea fields inspected during the survey were treated with chemicals for the management of the disease. The farmers in the areas practice cultural methods like sowing date adjustment, crop rotation, and weeding to manage the disease. They rotate chickpeas with cereal crops and legumes. Thus, 26 and 14 assessed chickpea fields were sown with cereals and legumes, respectively, during the previous growing season. The surveyed fields were dominated by vertisol soil types. In 65% of the fields surveyed, farmers practiced weeding. The surveyed chickpea fields differed in the growth stages; hence, 10%, 57.5%, and 32.5% of the fields were at vegetative, flowering, and pod-setting stages, respectively.

4.1. 2. Prevalence and incidence of chickpea Fusarium wilt

The survey results showed that Fusarium wilt disease was widely distributed and a very serious problem for chickpeas in the study areas. The disease was the most frequently occurring and devastating disease and was recorded at 100% prevalence in both districts during the 2022 main cropping season. The high prevalence of wilt disease shows that the disease poses a potential threat to chickpea production in the inspected areas. Wilt disease of chickpea is widely distributed and 100% prevalent in major chickpea-producing zones in north and south Gondar and East Gojam of Ethiopia, as reported by Abera *et al*. (2011).

In the current study, a wilt incidence ranging from 24.9% to 61.5% has been recorded. The highest (43.2%) mean FOC incidence was observed in Siyadebrna-wayu district, and the lowest (34.8%) incidence was recorded in Ensaro districts (Table 5). In a previous study of the 2006–2007 and 2007–2008 main cropping seasons by Abera *et al*.(2011), the mean incidences of chickpea Fusarium wilt were 34.2% and 34.1%, 37.9% and 35.4%, 34.7% and 28.8%,

34.7% and 28.8%, 34.7% and 28.8%, and 33.3% and 37.6%, respectively, in Gondar Zuria, Dembia, Libo-Kemkem, Fogera, Dejen, and Enemay, respectively. Damte and Ojiewo (2016) also found low to high wilt/root rot incidences of 0.0–83.4, 0.0–27.6, 1.3–19.8, and 0.0–16.3% in 2013/2014 and 1.0–81.9, 0.0–25.5, 3.0–13.9, and 1.0–21.5% in 2014/2015 in East Gojjam, Southwest Shoa, North Shoa, and West Shoa, respectively. Likewise, Ali and Terefe, (2021) observed Fusarium wilt of chickpea in all surveyed districts (Ensaro, Menz-Mama, Merhabete, Mojana-wedera, and Moretena-jiru) of the northern Shoa of Ethiopia, with the highest incidence (50.2%) in Ensaro and the lowest (34.3%) incidence in Mojana-wedera districts during the 2018–2019 growing season.

The high CFW incidence in Siyadebrna-wayu may be due to the crop growth stage and the poor cultural practices adopted by smallholder farmers in the area, including poor weed management practices. Most of the Siyadebrna-wayu district fields were at the pod setting stage, and a high CFW incidence (41.2%) was recorded at this growth stage. In addition, 45% of the Siyadebrna- wayu fields were weedy, whereas 25% of the Ensaro fields were weedy, and a high CFW incidence (45.8%) was observed in un-weeded fields.

The altitude of the study area was classified as <2300 and >2300 meter above sea level. High mean CFW incidence (40.3%) was observed in the fields at altitudes less than 2300 (<2300) than altitude greater than 2300 (>2300), which recorded a mean incidence of 38.7% (Table 5). In this study, it is clearly seen that Fusarium wilt of chickpea increased as the altitude decreased and vice versa. This might be due to the fact that lower altitudes receive a higher temperature, and the environmental conditions at this altitude are drier and warmer, which favor the disease as compared to higher altitudes, which have a lower temperature and a cooler environmental condition. This is in agreement with Mawar *et al*. (2021) who stated that soil-borne diseases were relatively more serious where the chickpea growing season was short, warm, and dry. Several other reports had similar findings to the current result (Assfaw and Negash, 2020; Ali and Terefe, 2021). However, contrary to the current finding, Negash (2021) reported a high level of CFW at high altitudes (above 2000 m.a.s.l.).

In the present study, a relatively higher mean CFW incidence was noted for the Desi type (39.7%) (Table 5) than the Kabuli chickpea type (37.5%). This result was in line with the

work of previous authors who found higher wilt/root rot incidence in Desi types of chickpea (Yimer *et al*., 2018; Bekele *et al*., 2021). In contrast, Ali and Terefe (2021) found a higher wilt incidence in Kabuli-type chickpeas. Yimer *et al*. (2018) and Bekele *et al*. (2021) explained that Desi-type chickpea seeds found in Ethiopia are often landraces without modern genetic improvements. However, Ethiopian Kabuli-type varieties are typically the product of organized crop improvement efforts, and their availability is more frequently associated with organized seed systems and the higher acceptance of new agricultural technologies. This might be the reason for their lower rates of disease.

During the survey, the crop was at the vegetative, flowering, and pod setting stages, and fields with plants at the pod setting stage had the highest mean disease incidence (41.2%) compared to the flowering (38.2%) and vegetative stages of the crop (33.8%) (Table 5). This indicated that the intensity of chickpea wilt disease increased while the crop was progressing to maturity. The observed difference in disease incidence might also be due to the increasing temperature that aggravates the disease spread since chickpea wilt disease is temperature-dependent and the density of the pathogen increases with time. Similar results were also reported by Navas-cortes *et al*. (2001), who indicated that the incidence of wilt was positively correlated with increasing soil temperature and the inoculum density of the pathogen.

Fusarium oxysporum f. sp. *ciceris* infects chickpea at seedlings as well as at flowering and pod-forming stages, with more incidence at the flowering and pod-setting stages if the crop is subjected to sudden temperature rise and water stress (Mawar *et al*.,2021; Maitlo *et al.*, 2014). This increment in incidence at the reproductive stage could be due to increased temperature (Negash, 2021). In contrast to the present study result, the highest Fusarium wilt incidence was observed at the vegetative stage, as Ramanamma *et al*. (2020) reported in India.

In addition, in the current study, a higher CFW incidence (39.2%) was observed on fields planted in late August than early September, which was 37.1% CFW incidence (Table 5. Similar work was done by Yimer *et al*. (2018) who found high wilt/ root rot incidence in the crops planted in late August. This might have been associated with the wet conditions present during the season that aggravate the disease (Yimer *et al*., 2018). Delaying sowing dates helps to escape root hypoxia, wilt/root rot, and foliar diseases (Tiwari and Meen, 2016; Yimer *et al.*,

2018). Late planting in October was associated with a reduced incidence of wilt/root rot diseases in farmers' fields (Bekele *et al*., 2021).

Besides the above independent variables, the CFW incidence level in the current study varied for different crops previously grown. Both cereals (wheat, teff, and barley) and legumes (Faba bean, lentil, and pea) were sown on the surveyed chickpea fields previously. High Fusarium wilt incidence (43.9%) was found on fields previously sown with legumes (Table 5). Analogous work was done by Negash (2021) in East Shewa Zone, Central Ethiopia. He found 53.7%, 49.6%, 49.8%, 53.2%, and 46.1% CFW incidence on previously chickpea, lentil, field pea, haricot bean, and faba bean-grown fields, respectively. This is due to the fact that previous crop residue serves as a source of inocula for the next cropping seasons (Srinivas, 2016; Aytenfsu *et al*., 2019; Bekele *et al*., 2021; Negash, 2021). In addition, in the absence of rotation or when chickpea was grown after other pulse crops, disease was more severe since the Fusarium wilt pathogen can colonize the roots of other pulse crops without developing external symptoms (Jimenez Diaz *et al*., 2015), thus increasing inoculum density within the soil. Agronomic practices can mitigate disease: rotating chickpeas with crops other than pulses and seed selection were all associated with a reduced incidence of wilt/root rot diseases in farmers' fields (Bekele *et al*., 2021).

The presence of weeds can affect the level of CFW incidence. During the current survey, 35% of the assessed fields were weedy. It was observed from the current study that the incidence of CFW in weedy fields was higher (45.8%) than in weeded fields (35.0%) (Table 5). The present result was in line with Ali and Tefere, (2021), Negash, (2021), and Yimer *et al*. (2018) studies. They found higher wilt/root rot on chickpeas grown on weedy fields. Weeds compete for soil moisture and nutrients that make the chickpea plant less vigorous and more susceptible to disease (Yimer *et al*., 2018; Misganaw *et al*., 2019). In addition, weeds may harbor pathogens from the preceding cropping season by acting as alternate hosts or as symptomless carriers and thus serve as a source of inoculum (Haware and Nene, 1982). This might be the reason for the higher wilt incidence in weedy fields. Indeed, previous studies have demonstrated that FOC can infect several weed species, like *Cyperus rotundus*, *Tribulus terrestris*, *Convolvulus arvensis*, and *Cardiospermum halicacabum* (Nene and Haware, 1980).

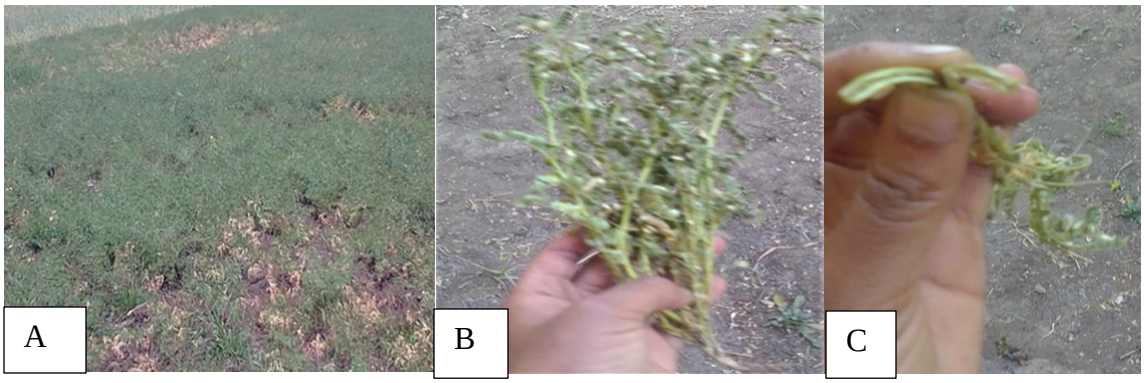

Figure 4. Status of chickpea fields in Siyadebrna-wayu and Ensaro districts. Chickpea Fusarium Wilt affected field in Siyadebrna-wayu district (A). Chickpea wilt-affected plant during the survey (B). Internal blackening of the stem caused by Fusarium wilt (C) (Photo: Kalkidan W.2022).

Table 4.Incidence of CFW on independent variables during 2022 growing season in N.Shoa

Variables	**Variable class**	**Mean CFW incidence (%)**
Altitude	<2300	40.3
	>2300	38.7
Chickpea type	Desi	39.7
	Kabuli	37.6
District	Siyadebrna-wayu	43.2
	Ensaro	34.8
Growth stage	Vegetative	33.8
	Flowering	38.2
	Pod setting	41.2
Planting date	Late august	39.2
	Early September	37.9
Previous crop	Legumes	43.9
	Cereals	36.3
Weeding practice	Weedy	45.8
	Weeded	35.0

4.1.3. Association of biophysical factors with chickpea Fusarium wilt

The logistic regression model indicated that independent variables studied in the survey varied in their level of association with CFW in the growing season (Table 6). District, growth stage of the crop, and previous crop were significantly ($P<0.05$) associated with CFW incidence. This finding is supported by Negash, (2021) who found highly significant ($P<0.0001$)

association of district, crop growth stage, and previous crop history with CFW incidence during the 2019 main cropping season in East Shoa, Ethiopia. In addition, Ali and Terefe, (2021) reported highly significantly (P< 0.001) association of district and crop growth stage with CFW incidence and severity in North Shoa Ethiopia, in 2018/19 growing season. Moreover, the finding of Bekele *et al*.(2021) showed significant (P< 0.01) association of chickpea wilt/root rot incidence with growth stage and previous crop during 2015 and 2016 survey in Amhara, Oromia and SNNPR region of Ethiopia.

In the present study, weeding practice had a highly significant (p<0.001) association with CFW incidence (Table 6). Similarly, Yimer *et al*. (2018) reported a highly significant (P <.001) association between weeding practices and chickpea wilt/root rot incidence and percent severity index (PSI) in the survey of the 2014/2015 cropping season in the mid-central and northern highlands of Ethiopia. High chickpea wilt/root rot incidence and percent severity index (PSI) were associated with un-weeded fields, while low wilt/root rot incidence and PSI were associated with reduced weed infestation, as these authors explained. And also, weed density was significantly associated with CFW incidence, according to Negash, (2021) report. However, Ali and Terefe, (2021) found a non-significant association between CFW incidence and severity and weeding practices.

On the other hand, altitude, chickpea type, and planting date were not significantly associated with CFW incidence, as the current result showed (Table 6). This finding was supported by Ali and Terefe, (2021), who reported a non-significant association between altitude and planting date and CFW incidence and severity. However, Yimer *et al*. (2018) demonstrated a significant (P < 0.01) association between chickpea type and planting date and chickpea wilt/root rot incidence and PSI. In addition, Bekele *et al*. (2021) reported a significant (P<0.01) association between chickpea seed type and planting date and wilt or root rot. Those authors found high wilt/root rot incidence on Desi chickpea type and early planting date compared to Kabuli and late planting date, respectively.

The probability of occurrence of a higher (>38%) chickpea Fusarium wilt incidence at altitude <2300 was 3 times greater than at altitude >2300. In addition, the probability of the occurrence of >38% CFW incidence in Siyadebrna- wayu district was 14 times higher than in Ensaro

district (Table 6). Moreover, the possibility of the occurrence of the highest (>38%) CFW incidence at the vegetative and flowering stages was 10 and 18 times, respectively (Table 6). Furthermore, the possibility of the occurrence of >38% disease incidence on late august planting date was 4 times higher than early September.

The non-significant association of altitude, chickpea type, and planting date with CFW in the present study might be due to the presence of suitable environmental conditions that favor Fusarium wilt development, and/or there might be different FOC races in the surveyed areas that enable the pathogen to widely infect the host regardless of the factors considered.

Table 5. Logistic regression model of chickpea Fusarium wilt incidence and odd ratio

Variables in the equation							
Variables	**Variable class**	**B**	**S.E**	**Wald**	**Df**	**Sig.**	**Exp(B)**
Altitude	<2300	1.116	1.575	.505	1	.449	3.053
Chickpea type	Kabuli	-459	1.164	.155	1	.694	.632
District	S/wayu	2.705	1.241	4.755	1	.029	14.960
Growth stage	Growth stage	-	-	4.004	2	.135	-
	Vegetative	2.366	2.239	1.117	1	.291	10.655
	Flowering	2.934	1.474	3.964	1	.046	18.810
Planting date	August	1.411	1.129	1.561	1	.211	4.098
Previous crop	Legumes	-3.251	1.438	5.108	1	.024	.039
Weeding practice	Weeded	-4.634	1.770	6.854	1	.009	.010
	Constant	1.820	1.569	1.346	1	.246	6.172

Variable(s) entered on step 1: Altitude, Chickpea type, District, Planting date, previous crop, weeding practice. Note: the last variable in each variable class were considered as reference in the model.

4.1. 4. Identification of chickpea wilt pathogen

The wilt-affected chickpea plants were identified in the field based on key symptoms of chickpea Fusarium wilt, like wilting, yellowing of leaves, and drying of plants. During the survey, yellowing and wilting of leaves, which resulted in the drooping of the entire plant or

its branches, and drying of chickpea plant symptoms were observed (Figure 6). In addition, when the roots of the infected plant split in two, brown to black discoloration of the xylem vessels was observed. Similar results were reported by Sonkar *et al.* (2014) who found a brown to black discoloration of internal vascular tissue (pith and xylem) upon inspection of roots longitudinally in both seedlings and adults. Moreover, Qureshi *et al*. (2021) observed brownish or blackish color in vertically opened infected chickpea plant stems (xylem). Further, Nikam *et al*. (2011) observed wilting symptoms on chickpea variety JG-62 after 25 days of inoculation with FOC. The initial symptoms were light yellow, drooping of leaves, and finally wilting of the host plant. Whereas, Patil *et al*.(2017) did not observe rotting of the outer surface of the wilted chickpea in the seedling stage; however, in the adult stage, they observed brown discoloration of internal tissues upon splitting vertically the discolored region downward as well as typical wilting.

The fungi causing chickpea wilt disease were identified morphologically by colony morphology (colony color and pigmentation) and conidia structure (macro and micro conidia) in the laboratory using Fusarium identification manuals (Nelson *et al*., 1994; Leslie and Summerell, 2000; Burgess *et al*., 1994). Colony diameter and the occurrence of chlamydospres were also studied. The colony characteristics of the FOC isolates isolated from chickpeas are presented in Table 7.

Colony diameter of the isolates were ranged in between 47.62 mm and 79.51 mm after 72 hr. of incubation on PDA medium at 25 ^{o}C (Table 7). The aerial mycelia of the isolates were white in color. All isolates formed abundant mycelia on PDA at 7 days (Figure 5A). The result of the present study is also confirmed by Jimenez Diaz *et al*. (2015), who reported that the aerial mycelium of FOC was at first white and cottony, but later it became cream or salmon in color or remained white. Similarly, Kumar *et al*. (2012) observed the white aerial mycelium of FOC isolates collected during the 2007–2009 extensive survey in the Eastern Plateau region of India. In addition, Qureshi *et al*. (2021) found white and creamy white aerial mycelium.

The current result showed that all FOC isolates did not form any pigmentation on agar (Figure 5B). Leslie and Summerell, (2000) also stated that *Fusarium oxysporum* produces no pigment at all. However, Golakiya *et al*. (2018) found brown, light brown, and pale yellow

pigmentation on 15 isolates of FOC collected from fifteen different locations in India. Qureshi *et al*. (2021) also observed reddish brown, light brown, light yellow, brown yellow, and pale yellow pigmentation of the colony on 14 FOC isolates collected from Khargone and Khandwa districts of India.

In the present study, the isolates had oval micro conidia and straight and slightly curved macro conidia (Figure 7). The micro conidia were formed without any septa (Figure 7D), while the macro had three septa (Table 7C). This result was supported by Thaware *et al.*(2017) who reported 0 septed micro conidia which were oval to cylindrical, and 3-5 septed macro which were sickle-shaped, curved, and fusoid. Soni *et al*. (2023) also reported the oval, cylindrical shape of micro conidia with 0–1 septa, whereas macro conidia were curved, typically sickle-shaped, with 1–8 septa. As Qureshi *et al*. (2021) reported, the number of septa in macro-conidia was mostly 1-4, micro-conidia had mostly no septum, and some were 0–1 with elongated and blunt ends with a sickle shape and a round to oval shape, respectively. In Cunnington, (2007) study, macro conidia were typically 3-septate, falcate to almost straight, and hooked apical cell and foot shaped basal cell. In addition, Ali and Terefe, (2021) reported elliptical (most of the FOC isolates) and oval (FOC-2, FOC-6, and FOC-14) micro conidia and straight macro conidia with 0-1 and 1-3 septa, respectively.

The chlamydospores were single round rough surface (Figure7E) and double round rough surface of the present FOC isolates (Figure7F). This result was similar with Ali and Terefe, (2021) who found single round rough surface of chlamydospores on 8 different FOC isolates. Kumar *et al.* (2012) also found singly formed chlamydospores.

The fungi were identified as *Fusarium oxysporum* f. sp. *ciceris* based on colony characteristics, macro- and micro conidia, and chlamydospore structure observed under a compound microscope using Fusarium identification manuals (Nelson *et al*., 1994; Leslie and Summerell, 2000; Burgess *et al*., 1994).

Table 6. Morphological characteristics of FOC isolated from chickpea

Isolates	Colony diameter (mm) 72hr. DAI	Mycelia		Macro conidia	Micro conidia	Chlamy dospores
		Surface	Reverse	No of septa	No of septa	
1	79.51	White	No pigment	3	No septa	SRR
2	72.00	‘’	“	‘’	‘’	‘’
3	73.96	“	‘’	‘’	‘’	‘’
4	65.84	“	‘’	‘’	‘’	DRR
5	47.62	‘’	‘’	‘’	‘’	‘’

Note; DAI= Days after inoculation; SRR=Single round and rough; Double round and rough surface

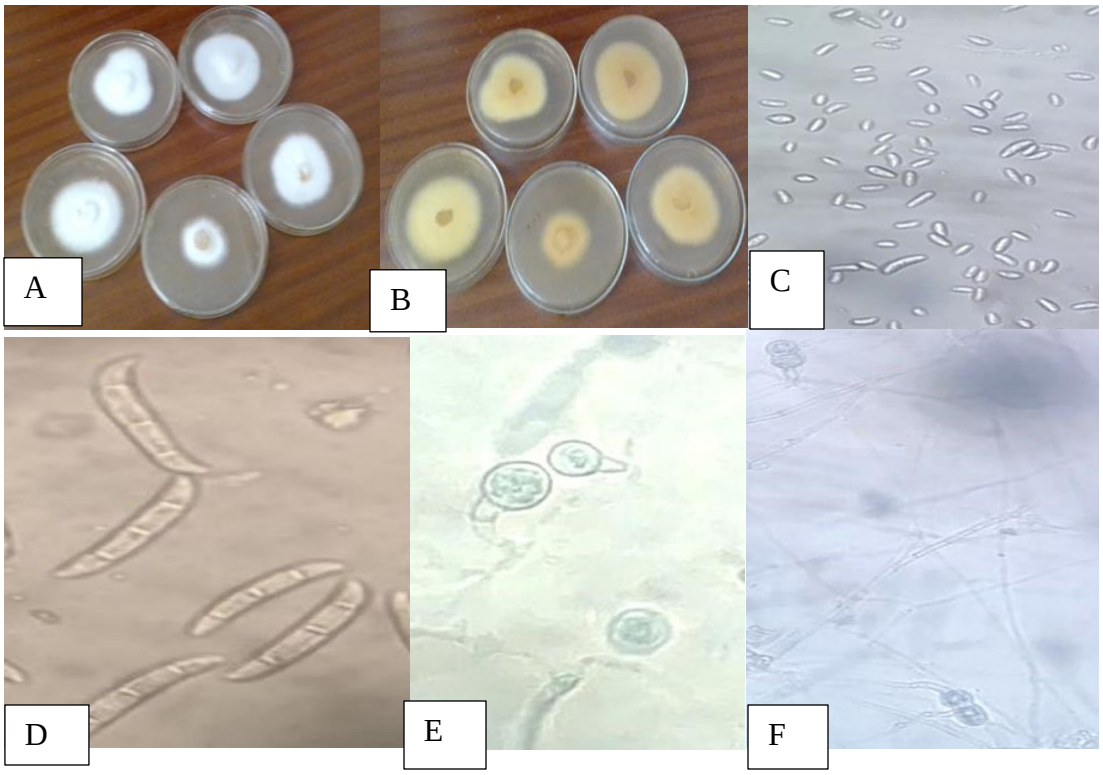

Figure 5. Colony morphology and spore of FOC; Surface (A), Reverse (B); Micro conidia (C) Macro conidia (D);Singly formed chlamydospores(E) and doubly formed chlamydospores(F) (Photo: Kalkidan W., 2022)

4.2. Pathogenicity test

The pathogenicity of the FOC isolates obtained from chickpea samples was tested in a pot experiment using the susceptible chickpea variety JG-62. The seedlings inoculated with FOC isolates exhibited wilting after 25 days of sowing. The result of the pathogenicity test revealed that all the isolates tested were pathogenic to chickpea. The initial symptoms produced were a light yellowing of seedling leaves that collapsed, lay flat on the ground, and finally dried the whole plant. The roots did not show any external rotting and looked apparently healthy, but when split vertically from the collar region downward, the roots showed a brown discoloration of the internal tissues. The symptoms produced during the pathogenicity test were identical to those observed on naturally infected, wilted chickpea plants in the field during the survey. These symptoms were exactly identical to those described earlier by Cunnington *et al*. (2007) and Jimenez-Diaz *et al*. (2015).

Re-isolation of fungi was made from the inoculated symptomatic chickpea plants grown in the lath house to test the pathogenicity of the FOC isolates. The re-isolated fungus had similar morphological characteristics as the original fungus. The fungus produced abundant white mycelia without any pigmentation on agar. It produced micro conidia and macro conidia. The micro were oval-shaped with no septation. While the macroconidia were straight and slightly curved with three septa. In addition, the chlamydospores were single round rough surface and double round rough surface.

The re-isolated fungus was confirmed to be *Fusarium oxysporium* f.sp. *ciceris*, which caused chickpea wilt, by comparing its morphological features with the original culture isolated from wilt-diseased chickpea plants using the Fusarium laboratory manuals (Nelson *et al*., 1994; Leslie and Summerell, 2000; Burgess *et al*., 1994), filling the requirement for Koch's postulates.

4.3. Evaluation of chickpea genotypes against Fusarium wilt

The analysis of variance revealed that there was significant difference ($P<0.000$) in the disease incidence among the screened genotypes. Out of the 14 chickpea genotypes and 2 check varieties tested, three genotypes (*DZ-2012-CK-0312, FLIP12-138c and ICCMABCD-21*) were moderately resistant; three moderately susceptible (DHERA, ICCU-10 and FLIP88-85c) and

the rest nine genotypes were susceptible with FLIP09-262c being the most susceptible one followed by FLIP12-342c and JG-62 (Table 8). The susceptible check JG-62 was completely wilted out within 25 days after sowing, indicating its highly susceptible response to FOC whereas; the resistant check DHERA showed 37.78% wilt incidence indicating its moderately susceptibility to the disease. However, none of the tested genotypes had complete resistance to FOC.

A similar study was carried out by Chaudhry *et al*. (2012) who screened 196 chickpea lines for their reaction to wilt disease in a wilt sick plot. In their research, none of the test lines was found immune to FOC. Arvayo-Ortiz *et al*. (2012) also evaluated lines of chickpea Hoga-012, Hoga-490-2 and Hoga-508, as well as the commercial cultivars Blanco Sinaloa-92 and Costa-2004 and reported that no variety showed resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*, isolated from chickpea fields of Sonora, Mexico. In Ethiopia Ayana *et al*. (2019) screened 21 chickpea varieties from Desi and Kabuli type against Fusarium wilt and reported that there were no highly resistant variety to the disease. On the other hand, Yadav *et al*. (2022) who studied sources of resistance to chickpea wilt reported that out of 40 genotypes tested, four genotypes were resistant, five were moderately resistant and the rest all were tolerant to highly susceptible to the disease. While, Hotkar *et al*. (2018) studied the reaction of chickpea entries to Fusarium wilt in 2014–2015 and 2015–2016 under field conditions and found 10 resistant, 18 moderately resistant, 18 susceptible, and 10 highly susceptible entries. In contrast to the current finding, Thaware *et al*. (2017) reported that of the 50 evaluated chickpea entries for resistance against Fusarium wilt, 6 entries were resistant.

Additionally, similar studies in Algeria were made by Khalifa *et al*. (2022) who reported that, out of 41 evaluated genotypes, 5, 16, 18, and 2 genotypes were resistant, moderately resistant, susceptible, and highly susceptible, respectively. In another study in Sudan, 20 chickpea genotypes were evaluated for their reactions to races 0, 2, and unidentified races, and they showed different rates of disease incidence (Mohamed and Mohamed, 2020). Assfaw *et al*. (2019) reported the reactions of the chickpea varieties Arerti and Shasho (resistant), Habru (moderately resistant), and local Dz-10-4 (susceptible) to chickpea Fusarium wilt.

Table 7. Mean values of disease incidence and reaction of the tested genotypes

No	Genotypes	Wilt incidence (%)	Reaction
1	FLIP09-262c	100A	S
2	FLIP12-342c	100A	S
3	JG-62	100A	S
4	DZ-2012-CK-0280	78.3B	S
5	FLIP13-379C	78.3B	S
6	DZ-2012-CK-0027	72.2BC	S
7	FLIP12-07c	72.2BC	S
8	FLIP-13-344C	70.0C	S
9	ICCU-16107	62.2D	S
10	DZ-2012-CK-2074	53.3E	S
11	DHERA	37.8F	MS
12	ICCU-10	35.6F	MS
13	FLIP88-85c	27.8G	MS
14	DZ-2012-CK-0312	20.0H	MR
15	FLIP12-138c	20.0H	MR
16	ICCMABCD-21	20.0H	MR
17	CONTROL	0.0I	-
CV		9.8	
LSD		6.3	

NOTE; S=susceptible, MS= moderately susceptible, MR= moderately resistance, R= resistant LSD =Least significant difference at $P \leq 0.05$, CV = Coefficient of variation. Means followed by similar letters in a column are not significantly different at 5% LSD.

5. SUMMARY CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusion

Chickpea is an annual legume crop which is mainly grown as source of human food and animal feed. In addition, chickpea returns significant amount of nitrogen to soil and improves soil fertility and break the disease cycles of important cereal pathogens and for this main reason, it is used in rotation with several cereals like teff and wheat on heavy soils. Chickpea production and productivity is affected by several biotic and abiotic factors. Chickpea Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceris* is one of the most important biotic constraints of chickpea production. Use of resistant chickpea varieties is one of the best approaches in the management of the disease, as it is cheaper and safer to environments. This study was conducted to assess the chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris)* intensity in association with biophysical factors in two selected districts of North Shoa, Ethiopia, and evaluate chickpea genotypes against the pathogen to improve the productivity of chickpea in North Shoa, Ethiopia through the use of appropriate chickpea Fusarium wilt management methods.

In this study, none of the inspected farmers' field were remained free from the wilt disease. However, there was variation in disease magnitude across districts. Higher FOC incidence (43.2%) were observed at Siyadebrna wayu district, while lower incidence (34.8%) were recorded at Ensaro district. High mean CFW incidence was recorded on variable classes such as lower altitude(<2300), pod setting crop growth stage, desi type chickpea, fields previously sown with legumes and weedy fields. District, crop growth stage, fields previously sown with legumes and weedy fields are the biophysical factors that have strong association with chickpea Fusarium wilt incidence, and had significant contribution to CFW epidemics development. Growers in the study area did not practice disease management techniques to control CFW, except late sowing (sowing date adjustment), weeding practices and crop rotation.

The wilt causing pathogenic fungi was isolated from wilt infected roots of chickpea. The wilt causing fungi in chickpea was identified as *Fusarium oxysporum* f. sp. *ciceris* using field symptoms and morphological features of the fungi (colony morphology and spore structure)

using Fusarium identification manuals. Generally, the field survey concluded that the disease is highly distributed and is a very important in the study areas

A lath house experiment established to test the reaction of chickpea genotypes/varieties against FOC. All the tested genotypes/varieties showed symptoms of Chickpea Fusarium wilt (CFW) disease at different assessment periods. Therefore from this finding, it can be concluded that there was no highly resistant chickpea genotypes /variety against CFW disease among the test genotypes/varieties. The tested genotypes ranges from moderately resistant to susceptible reaction to the pathogen. Out of the 14 chickpea genotypes and 2 check varieties tested, three genotypes (*DZ-2012-CK-0312, FLIP12-138c and ICCMABCD-21*) were moderately resistant and nine genotypes were susceptible with G9 being the most susceptible one.

5.2. Recommendation

Based on the results of this study, the following recommendations were made.

- Designing comprehensive and effective CFW management options that target the important biophysical factors, along with other crop husbandry practices, is essential to reduce the disease pressure
- Conducting a comprehensive field survey is required to generate a better conclusion about the association of pertinent biophysical factors with disease intensity.
- In addition, it is important to find out possible disease management options that include introducing of resistant varieties, good drainage practice and crop rotation (chickpea-cereals), using disease free seeds, screening effective systemic fungicides, and using effective bio-agents in the study areas.
- The current study was based on only morphological identification of the causal pathogen. However, as there are several fungi associated with chickpea wilt disease, it is essential to do further research on molecular identification of the fungus associated with chickpea wilt.
- It would be better to repeat the late house experiment, for one or two times and further tests under field conditions to come up with sound recommendations.

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APPENDICES

Table 1 : Logistic regression model

Omnibus Tests of Model Coefficients

		Chi-square	Df	Sig.
Step 1	Step	26.926	8	.001
	Block	26.926	8	.001
	Model	26.926	8	.001

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	27.622[a]	.490	.658

a. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

Classification Table[a]

			Predicted		
			disease incidence		
	Observed		38 and less than 38	above 38	Percentage Correct
Step 1	disease incidence	38 and less than 38	14	3	82.4
		above 38	3	20	87.0
	Overall Percentage				85.0

a. The cut value is .500

Table 2: ANOVA for genotypes screening

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	16	47586.8	2974.18	203.83	<0.0000
Error	34	496.1	14.59		
Total	50	48082.9			
Grand mean	39.029				
CV	9.79				

Figure 1: Picture during field observation and interview

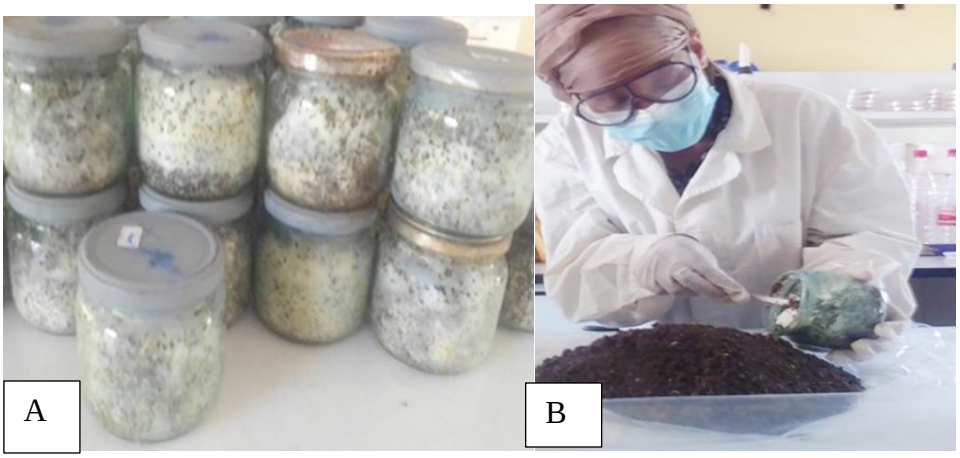

Figure 2. Mass Production of FOC isolates

Growing of FOC isolates isolated from collected wilted chickpea plants (A), mixing of growing FOC isolates with sterilized soil mix (B)

Figure 3: Pots used in the lath House for pathogenicity test

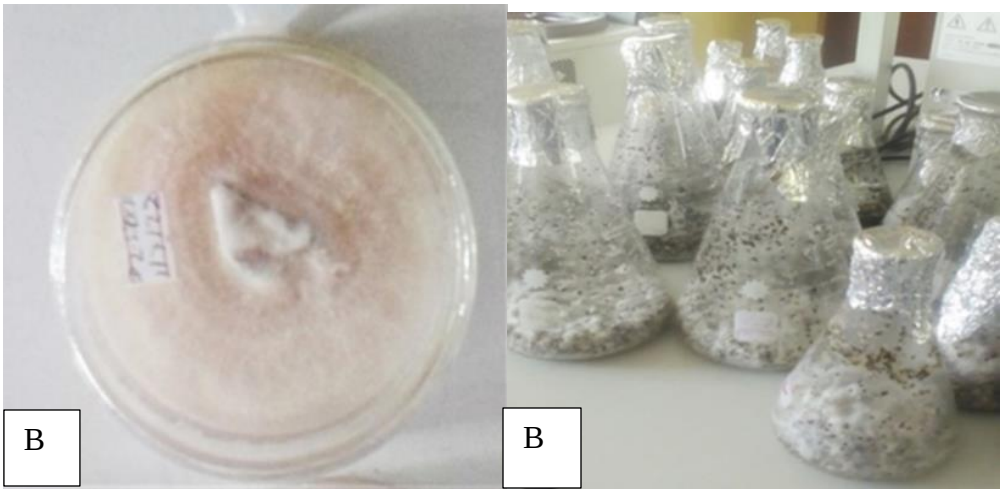

Figure 4. Mass production of FOC for genotype screening
Pure culture of FOC (A), growing of FOC pathogen on sand maize meal medium (B)

Figure 5: Picture during genotype screening

BIOGRAPHY SKETCH

The author was born at Shebel-Berenta district, East Gojjam Zone, Amhara Regional State, on 10 October 1997. She attended her elementary education at Addis Zemen Primary School and secondary education at Entoto abmba secondary school. Then, she attend preparatory education at Yekatit 12 Preparatory School. Upon passing the Ethiopian Higher Education Entrance Examination in 2017, she joined Debre birhan University in 2017 and graduated with BSc degree in Plant Science in 2019. After her graduation, she joined Debre birhan University and served as Graduate Assistant (GA) for one year from September 2019 until August 2020. Then she joined Hawassa University in 2021 to pursue her MSc Crop Protection (Plant Pathology).